Proceedings of The Forum Sessions
Forty-fourth Clinical Congress of the American College
of Surgeons, Chicago, Illinois
October, 1958

Surgical Forum

VOLUME IX

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AMERICAN COLLEGE OF SURGEONS
Chicago, Illinois, 1959

LIBRARY OF CONGRESS CATALOG CARD NUMBER 51 2646

PRINTED IN U. S A.

Foreword

The Forum on Fundamental Surgical Problems, authorized by the Board of Regents of the American College of Surgeons in 1941, has grown steadily in the intervening years. It has become a true forum before which the young surgeons of this country present annually the fruits of their experimental studies. Each year the competition for a place on the program becomes greater, and the task of the Forum Committee in selecting the program from the increasingly large number of abstracts submitted more difficult. The papers presented and included in this ninth annual Surgical Forum represent fundamental contributions of which all American surgeons can justly be proud. No volume reflects better the current progress of surgery. It has become an essential for all interested in such progress.

The engagement of so many young men in investigative work is playing an important role not only in molding the future of surgery itself but also in creating an inquiring, stimulating atmosphere in the medical schools and hospitals of this country. The Forum Committee is happy to have had a small part in the ever expanding interest of these young surgeons in basic laboratory and clinical research. It and the College present with pride this volume of carefully prepared and nicely illustrated papers dealing with various problems in general and special surgery.

Deep appreciation is expressed to Helene Coleman for her excellent editing of the manuscripts.

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Surgical Forum IX

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A METHOD TO STUDY ACUTE EXPERIMENTAL HEMORRHAGE *

VERNON MONTGOMERY, JEAN BLAVIER DALTON JENKINS AND HENRY SWAN

It is unfortunate that to date no standard experimental method has been widely accepted to study the effect of hemorrhage or hemorrhagic shock which is based upon blood loss alone Any technique which involves controlled hypotension[1 2] cannot be used to determine the efficacy of any therapeutic agent in experimental shock when that agent itself has an effect upon arterial pressure The major stumbling block in establishing such a method has been the variability and unpredictability of the response of the experimental animal to measured amounts of blood loss

The purpose of this paper is to analyze a method we have previously presented[3] involving a rapid arterial hemorrhage of a measured proportion of the blood volume of the splenectomized healthy nembutalized dog particularly in terms of the relationship between the volume of the hemorrhage and mortality Submission of the data to probit analysis provides a method of utilizing this preparation for evaluation of therapeutic techniques or other experimental variants

METHOD

Data are presented here on 74 mongrel dogs weighing 10 kg or more. All animals were splenectomized under aseptic conditions Following this procedure, all animals were placed in isolated cages and fed the same diet for a period of at least 30 days Animals which became ill during this period were treated by a veterinarian until the animal was either cured or dead We feel reasonably sure that these animals at the time of the experiment, were in good health and in a steady nutritional state It is our opinion that these details are of paramount importance

Before bleeding the animal was anesthetized with intravenous pentobarbital The right femoral vein and the left femoral artery were cannulated using aseptic technique The mean femoral arterial pressure was continuously monitored by means of a mercury manometer The animal was left in such a state for at least one hour or until his arterial pressure had not varied more than 10 mm Hg for a 30 minute period At this time the animal s blood volume was determined by RIHSA from a single 10 minute sample Within 15 minutes of the blood volume determination the bleeding began An animal was bled 35 39 42 or 45% of its measured blood volume through the femoral arterial cannula The bleeding time was from 3 to 5 minutes Following this the animal was observed for several hours If it survived this

* From the Halsted Experimental Laboratory University of Colorado School of Medicine Denver Supported by a research contract (DA-49-007 MD 572) with the Surgeon General United States Army This work was done partially during the tenure of an Advanced Research Fellowship of the American Heart Association

initial period it was returned to its cage To be classified as a survivor, the animal had to be alive 7 days later

Included in our outline of proposed experiments is a study of the influence of hypothermia on mortality following hemorrhage Since hypothermic animals must be respired it was necessary to determine whether artificial intermittent positive pressure respiration had an influence on mortality following hemorrhage in the normothermic animal Therefore Group 2 contains 15 animals which were treated just as Group 1 with the exception that the former were artificially respired for 4 hours following the bleeding

RESULTS AND DISCUSSION

Table 1 summarizes the mortality data from both groups If these data are plotted as mortality against the accumulated per cent of blood volume hemorrhage a typical sigmoid or S shaped curve is seen This is the pattern when any measurement that falls in a normal or Gaussian distribution is plotted as per cent of total against the measurement Since curvilinear relations are difficult to handle statistically a method of rectifying our data was sought

Table 1 Mortality Rate with Various Amounts of Acute Arterial Hemorrhage

HEMORRHAGE (PER CENT OF BLOOD VOLUME)	GROUP 1		GROUP 2	
	MORTALITY	NUMBER OF DOGS	MORTALITY	NUMBER OF DOGS
35	0	34		
39	25	4	12	8
42	45	11	71	7
45	89	9		

Bliss[4] has shown that such a sigmoid curve can be made linear by plotting the log of one measurement against the probit of the other (A probit is defined as the normal deviate of the Gaussian curve plus 5)

Figure 1 shows the data from Group 1 plotted in this manner—the log per cent of blood volume hemorrhage on the abscissa against the probit mortality on the ordinate The equation for this line is $Y = 54\ 63 + 36\ 86\ X$ where Y is the mortality in probits and X is the log of the per cent hemorrhage The Chi square test for fit of these data around the calculated regression line yields $50 > p > 30$ This value indicates that the observed variation of points around this line is very likely the result of random sampling and thus lends credence to the regression line The curved broken lines are the calculated 95% confidence limits One would expect further samples of the mortality of animals due to hemorrhage to fall within these confidence limits 19 times out of 20

The utility of this type of analysis can be exemplified by data from Group 2 Figure 2 shows the same calculated lines given in Figure 1 The circles are a plot of the data from Group 2 These points f-ll l within the confidence limits of the regression line This fact leads that the variable

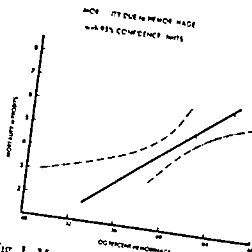


Fig 1 Mortality in probits at various levels of per cent of blood volume hemorrhage The solid line is the calculated mean regression line The broken lines indicate the 95% confidence limits The dots represent the means of Group 1

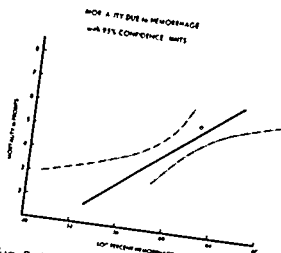


Fig 2 Mortality in probits at various levels of per cent of blood volume hemorrhage The lines are those of Figure 1 The circles represent the means of Group 2

of artificial respiration does not influence mortality due to hemorrhage

Another useful aspect of the probit analysis can be illustrated from Figure 1 By definition the LD 50 bleeding volume will lie at the intercept of probit 5 with the regression line and the 95% confidence limits will define the limits of such a volume Inspection of the chart and reference to the logarithm table shows this figure to be 10 to 13% In other words there are approximately 19 chances in 20 that 50% of a group of animals bled in this range will die

SUMMARY

1 A standardized method for producing acute arterial hemorrhage in the dog has been described based on the rapid withdrawal of a specific per cent of the measured blood volume

2 The relation between the volume of hemorrhage and mortality has been studied and analyzed

3 Probit analysis is offered as a useful method of utilizing these data for experimental designs

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THE EFFECT OF NOREPINEPHRINE (LEVOPHED) ON SURVIVAL IN STANDARD OLIGEMIC SHOCK *

JAMES C GRIFFIN, JR, WATTS R WEBB, SAE S LEE, JAMES D HARDY AND JOHN M MCRAE, JR

In the effort to find effective ways of treating resistant shock, vasopressors have been used in recent years in numerous clinical and experimental situations. In fact it has become almost routine, if blood is ineffective or not available to resort to the use of vasopressors Various experimental studies have indicated that vasoconstrictors may be deleterious in hemorrhagic or traumatic shock [1, 2] A recent study by Clove and his associates showed a mortality rate of 65% in dogs treated with norepinephrine for hemorrhagic shock, with only 33% in the controls As many of these studies did not closely simulate the usual clinical use of norepinephrine, the following experiment was designed to approximate the clinical utilization of Levophed in hemorrhagic shock

METHOD

Healthy mongrel dogs of both sexes weighing between 10 and 20 kg, fasted overnight, were anesthetized with intravenous thiopental and heparinized with 10 mg/kg of heparin A plastic catheter was placed in the femoral artery and attached by a Y tube to a reservoir and a mercury manometer Over 30 dogs were utilized to develop a satisfactory method of achieving a standard proportionate reduction of the circulating blood volume, thus reducing the animals to a constant physiologic state The blood pressure was reduced over a period of approximately 4 minutes to 20 mm of Hg and maintained at this level for exactly 20 minutes The arterial reservoir was then clamped for 90 minutes, at the end of which time the blood was reinfused

During this 90 minute period the 22 experimental dogs received an infusion of norepinephrine in saline (16 μg/cc) sufficient to adjust the mean arterial blood pressure at approximately 80 to 9 mm of Hg During the development of the experimental methods it was noted that if the blood pressure was maintained above 90 mm of Hg the procedure was invariably fatal usually during the period of administration of norepinephrine Following

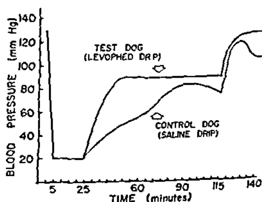


Fig 1 Graph showing typical blood pressure responses with and without norepinephrine

* From the Department of Surgery, University of Mississippi Medical Center, Jackson

Supported by U S Army Contract Number DA-49 007 MD627

this observation the blood pressure was maintained above 80 but below 90 mm of Hg and this resulted in a more satisfactory procedure The 22 control animals received intravenous saline in comparable amounts and at the same rate as the paired experimental animals The amount of saline administered average 15 cc /kg body weight in both groups

Serial hematocrits were obtained and bleeding volumes were recorded These animals received no antibiotics or further treatment after reinfusion of the blood All animals which lived 24 hours were considered survivals

RESULTS

The mortality rate in the control series was 50% while that in the experimental study was 18% Bleeding volumes from both groups showed no significant difference, being 16 3 cc in the treated dogs and 17 7 cc in the controls The serial hematocrits showed approximately equal changes in both groups with a fall in the hematocrit in each until restitution of the stored blood was reached

Table 1 Hypovolemic Shock

	DOGS	HEMATOCRIT	BLEEDING VOLUME	EARLY DEATHS	LATE DEATHS
CONTROL	22	44	47 7	7	4
LEVOPHED	22	46	46.3	3	1

Table showing comparative data on the two series Early deaths are those during hypovolemia and late deaths are those after reinfusion of the blood but within 24 hours P< 025

Four of the 11 deaths in the control animals occurred after conclusion of the experiment with 7 dying during the period of hypovolemia On the other hand 18 of the 19 levophed animals that survived the 90 minute period of oligemia were permanent survivors Three of the 4 mortalities that occurred in the treated series occurred during the infusion of norepinephrine These 3 showed a similar syndrome with a slowing heart wide bounding pulse and rapid demise thought to be a cardiotoxic effect of excess norepinephrine

DISCUSSION

The result of this investigation demonstrates that the careful use of norepinephrine in hemorrhagic shock can be of value prior to the restablishment of normal blood volume Lansing and Stephenson[4] reported that in the period immediately following hemorrhagic shock, norepinephrine has a direct beneficial effect on the heart and maintenance or increasing the cardiac output is the beneficent effect rather than increasing the peripheral resistance In view of the excellent studies demonstrating that irreversibility in hemorrhagic shock can be prevented by perfusion of the liver or intestines[5, 6] it must be recognized that any diminution of blood flow to vital areas may well be detrimental Thus our results are not in contradiction to the concept that an excessive increase of vasoconstriction is harmful during hemorrhage, as our experience likewise demonstrates that excessive norepinephrine can prove

disastrous In addition if *in vivo* coagulation as suggested by Crowell[7] is one of the significant lethal factors in shock this has been obviated by the large doses of heparin used in this and most other experimental studies The effects of norepinephrine might be partially reversed by preventing coagulation of blood trapped within the constricted capillary beds

SUMMARY

In summary, the results of this study indicate that norepinephrine when used carefully and in minimal dosages can be of value in hemorrhagic shock Our observations suggest higher dosages may well be deleterious This study however, cannot be considered comparable with clinical experience until it has been repeated without anticoagulants

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THE SECRETION OF EPINEPHRINE NOR EPINEPHRINE AND CORTICOSTEROID IN THE ADRENAL VENOUS BLOOD OF THE HUMAN *

DAVID M HUME AND C COOPER BELL JR

During the course of abdominal laparotomy in 8 patients the left adrenal vein was exposed and cannulated by a modification of the technique described by Hardy and Turner[2] Samples were drawn into a heparinized syringe at timed intervals and the constriction of the vein was released between samples to prevent the development of increased venous pressure with distension and engorgement of the gland Since all the effluent of the adrenal vein was col lected over a measured period of time the adrenal blood flow was recorded for each sample The samples were analyzed for epinephrine, nor epinephrine and 17 hydroxycorticosteroid content and results were expressed in terms of the minute output of these substances

METHOD

The plasma content of the free 17 hydroxycorticosteroids was determined by the method of Nelson and Samuels[7] The conjugates were estimated by deter

* From the Department of Surgery Medical College of Virginia Richmond Supported in part by the United States Air Force under contract number AF 41 (657) 169 monitored by the School of Aviation Medicine USAF Randolph Air Force Base Texas and by a Grant in Aid from the Commonwealth Fund

mining the total corticosteroid content of the plasma after hydrolysis by the method suggested by Steenburg[10] and measuring the corticosteroids by a modification of Peterson's[9] modification of the Silber Porter method The free corticosteroids were then subtracted from the totals to give the conjugates and duplicates were run on the free steroids by the Nelson Samuels method[7] The plasma epinephrine and nor epinephrine were determined by a modification of the method of Weil Malherbe and Bone[12]

RESULTS AND DISCUSSION

The additional dissection needed to expose the adrenal vein and the time required to withdraw the samples added about 20 minutes to the operative time No untoward results were noted as a consequence of the additional procedure and all patients in this series made uneventful recoveries

The results are summarized in Table 1 All patients received premedication consisting of demerol 75 to 100 mg and atropine or scopolamine 0 4 mg the hour before operation and nembutal 100 mg 2 hours before operation The patients in the first 5 cases all had peptic ulcers either gastric or duodenal and all were subjected to subtotal gastrectomy A vagotomy was also done in four of the five cases The same anesthetic agents were employed in these five cases consisting of pentothal and nitrous oxide combined with intravenous demerol and some type of muscle relaxant The total amounts of these substances used during the operation are listed in Table 1 There were 4 males and 1 female in this group ages ranging from 13 to 73 years The patient in case 6 was subjected to a total gastrectomy splenectomy partial pancreatectomy and resection of a portion of the transverse colon for carcinoma of the stomach with extension into the surrounding structures Continuous epidural anesthesia was employed but supplementation with pentothal nitrous oxide-demerol muscle relaxant anesthesia was necessary The patient in case 7 had a subtotal gastrectomy for duodenal ulcer under continuous epidural anesthesia with administration of demoral intravenous nembutal and desoxyn The patient in case 8 had an aortic bifurcation graft with exploration of the renal arteries for thrombosis of the terminal aorta and hypertension Hypothermia was used in this case because it was planned to occlude and open the renal arteries The results were as follows

1 **Adrenal venous blood flow (AVBF)** The AVBF varied from 2 8 cc/minute in the patient undergoing the most traumatic procedure to 54 6 cc/minute in the patient undergoing a subtotal gastrectomy Four points on AVBF are emphasized *a*) AVBF must be measured rather than to rely on the concentrations of the hormones per unit of adrenal blood *b* The minute output of corticosteroid is not necessarily directly proportional to the AVBF Although the greatest corticosteroid output occurred in the patient with the greatest AVBF the patient with the second greatest AVBF had the lowest corticosteroid output The patient with the least AVBF had the greatest epinephrine output and other patients with widely divergent AVBF secreted very comparable quantities of epinephrine *c* Although it has been shown by experiments in the dog that the AVBF in any particular case may vary directly with the blood pressure (Hume and Nelson[4]) blood pressure alterations do not account for the widely different AVBF in this group of patients *d* Anatomical variations may permit accessory venous channels to carry off part of the adrenal effluent although cursory examination revealed

Table 1

PT	AGE	SEX	WT KG	DIAG	OPERATION	ANES	B P	TIME OF SAMPLE	AVBF CC/MIN	FREE 17 OH C S γ/MIN	CONJ 17 OH C S γ/MIN	POST ACTH FREE C S γ/MIN	EPI γ/MIN	N E γ/MIN
1) C E	46	F	39.0	Duodenal Ulcer	Vagotomy, Sub total Gastrectomy	P 1.125 g Nitrous D 40 mg C 21 mg A 30 mg	110/60	2 hours	6.3	23.1	—	—	0.013*	0.163*
2) R C	53	M	58.2	Gastric Ulcer	Vagotomy, Sub total Gastrectomy	P 0.9 g Nitrous D 100 mg C 39 mg A 60 mg	110/80	3 hours	6.0	70.3	—	60.4	0.208	0.216
3) J A	57	M	63.6	Duodenal Ulcer	Vagotomy Sub total Gastrectomy	P 1.15 g Nitrous D 110 mg C 33 mg	125/70	3 hours	9.5	80.0	0.0	—	0.197	0.112
4) W R	43	M	77.3	Gastric Ulcer	Sub total Gastrectomy	P 0.75 g Nitrous D 70 mg C 21 mg	130/85	1½ hours	54.6	123.0	0.0	143.0	0.372	0.0
5) K K	73	M	60.5	Gastric Ulcer	Vagotomy, Sub total Gastrectomy	P 1.6 g Nitrous D 100 mg T 42 mg A 50 mg	185/90	4½ hours	5.0	27.2	0.0	61.2	0.214	0.081

Table 1 (continued)

PT	AGE	SEX	WT KG	DIAG	OPERATION	ANES	B P	TIME OF SAMPLE	AVBF cc/MIN	FREE 17 OH C S γ/MIN	CONJ 17 OH C S γ/MIN	POST ACTH FREE C S γ/MIN	EPI γ/MIN	N E γ/MIN
6) R G	52	M	72 7	Carcinoma of stomach with extension	Total gastrectomy Splenectomy Partial Pancreatectomy Colectomy	Continuous epidural P 0 5 g Nitrous D 80 mg A 80 mg Des 10 mg	$\frac{110}{65}$	1¼ hours	2 8	17 1	—	20 7	0 923	0.311
7) B A	29	M	46 8	Duodenal Ulcer	Sub total Gastrectomy	Continuous epidural D 70 mg N 100 mg Des 65 mg	$\frac{130}{80}$	1/ hours	23 0	7 1	0 0	—	0.231	0 119
8) M J	30	F	13 2	Thrombosis aorta hypertension	Aortic Bifurcation Graft	Hypothermia P 0 5 g Nitrous D 90 mg C 18 mg A 10 mg	$\frac{160}{100}$	1/ hours	1 0	10 7	0 0	—	0 211	0.211

• Flow had slowed when this sample was taken and the corticoid value had dropped sharply, so these values are not accurate

A anectine AVBF adrenal venous blood flow B P blood pressure at time samples taken C curare D demerol Des desoxyn Epi epinephrine
N nembutal (I V) N E nor epinephrine P pentothal T tubocurare 17 OH C S 17 hydroxycorticosteroid
The time of the sample refers to the time the sample was taken after the beginning of the operation

none in these cases The epinephrine output in six of eight cases was very similar, however, and case 6, with the highest epinephrine, had the lowest AVBF, suggesting that most of the adrenal output was being collected

2. 17-hydroxycorticosteroid output. Corticosteroid output in the first five cases varied from 23 1 μg/minute to 123 0 μg/minute, averaging 64 7 μg/minute Hardy and Turner[2] found the corticosteroid output to be 28 μg/minute The corticosteroid output in an individual patient tended to be fairly constant from sample to sample Values listed in Table 1 are averages of all the samples in each case

3. Conjugated corticosteroid output. It was reported by Hardy and Turner[2] that a considerable quantity of the adrenal corticosteroid output was in the form of glucuronide conjugates As the conjugates are inactive, and conjugation of the corticosteroids ordinarily takes place in the liver, this seemed difficult to explain In the present study conjugates were determined on samples from 5 patients, using two different techniques No conjugates were found, all 17 hydroxycorticosteroid secretion being in the form of the free compound Our results thus differed considerably in this regard from those reported by Hardy and Turner[2]

4. Effect of ACTH. In 4 cases, after collecting control samples, ACTH was administered intravenously in a single dose of 25 units Another sample was then collected at intervals varying between 3 and 10 minutes after the ACTH was given No significant increase in secretion was observed in three of four cases, suggesting that the adrenal was already working at maximum capacity In case 5 there was a marked increase in 17 hydroxycorticosteroid secretion following ACTH, which would suggest that in this case the adrenal was not secreting maximally prior to ACTH

5. Epinephrine and nor epinephrine levels. Discounting the values in case 1, which were inaccurate due to technical difficulties, the output of epinephrine varied between 0 197 and 0 923 μg/minute, with an average of 0 337, while that for nor epinephrine varied between 0 0 and 0 341 μg/minute with an average of 0 115 There was a tendency toward a somewhat greater excretion of epinephrine than nor epinephrine in spite of the fact that pentothal and nembutal anesthesia tend to suppress epinephrine secretion The values for epinephrine would certainly have been much higher had ether anesthesia been used, and studies are now under way in patients receiving this agent That the highest level of epinephrine secretion was seen in the patient having the most traumatic procedure, in spite of the fact that this patient had the lowest AVBF, may have been because this patient had a considerable amount of blood loss and tended to drift into shock Shock is a stimulus to epinephrine secretion in the dog (Hume[6]) This patient also received a relatively small amount of pentothal compared to some of the other patients and this may have led to decreased inhibition of epinephrine secretion

6. Epidural anesthesia. Two patients in this series were operated upon under continuous epidural anesthesia The corticosteroid response in both these patients was below that seen in any of those receiving general anesthesia This seems particularly significant in case 7, where the AVBF was 23 cc /minute, and the corticosteroid output only 7 1 μg/minute These results are consistent with those found in the dog (Hume[3]), where cord section abolished the adrenal cortical response to injury below the level of section The adrenal medullary response in case 6 was the greatest seen in any patient,

and suggests that the epidural anesthesia was not effective in blocking all stimuli to the medulla.

7. **Hypothermia.** One of the patients in this series was operated upon under hypothermia, and had a body temperature of 30°C when the samples were taken It has been shown in the dog (Egdahl *et al.*[1]) that hypothermia decreases the output of corticosteroid from the adrenal and (Hume *et al*[5]) that it depresses epinephrine, nor-epinephrine, and ACTH secretion as well The corticosteroid output in case 8 appears to be reduced, while the epinephrine and nor epinephrine secretions are similar to those of the other patients At 30°C the depression of the adrenal is just beginning, becoming much more marked at 28°, and it is probable that depression of the epinephrine secretion would have been seen had the temperature been somewhat lower.

8. **Blood and urine changes.** In case 3 peripheral blood corticoid levels and urinary epinephrine, nor epinephrine, and corticosteroid secretion were determined, as well as adrenal venous blood samples. The urinary epinephrine and nor epinephrine were determined by the method of Von Euler and Floding[11], the urinary corticosteroids by the method of Peterson.[8] In Figure 1 the blood corticosteroid response to a test dose of ACTH and to the operative trauma are shown It may been seen that at the time the adrenal sample was being withdrawn there was a high peripheral corticoid level as well In Figure 2 the urinary values are charted. No intraoperative urinary samples were obtained in this case. It may be noted that there is an increase in both epinephrine and nor-epinephrine excretion in the postoperative period, and that more nor epinephrine is excreted than epinephrine, the main contributor being the nerve endings

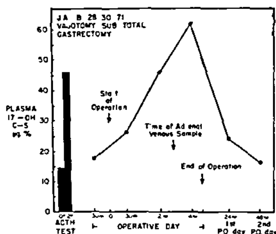


Fig 1 Plasma corticosteroid levels in case 3 taken 30 minutes prior to the start of the operation at intervals during the operation and on the first and second postoperative days It may be seen that there was a high plasma corticosteroid level at the time the adrenal venous sample was taken, and that the peripheral levels fell to normal by the second postoperative day The response to 25 units of ACTH is shown in the two columns to the left

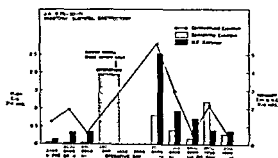


Fig 2 Urinary corticosteroid, epinephrine, and nor epinephrine excretion in case 3 Four hourly urine collections were made, and the values are given in micrograms per 4 hours The samples from 0800 to 2000 on the day of operation were not obtained It may be seen that there was a marked increase in corticosteroid, epinephrine, and nor epinephrine excretion following the operation In all samples save one there was a greater excretion of nor epinephrine than epinephrine

CONCLUSIONS

1. The adrenal venous blood flow was found to vary from 2 8 cc./minute to 54.6 cc /minute in the 8 patients investigated

2. The 17-hydroxycorticosteroid output varied from 23 1 to 123 0 μg/minute, with an average of 64 7 in the five patients receiving pentothal nitrous oxide-demerol muscle relaxant anesthesia

3 The corticosteroid output was reduced with epidural anesthesia or with hypothermia.

4. The administration of ACTH intravenously during the operation did not produce an increased corticosteroid output in three of four cases, ap parently because the adrenal was already secreting at maximal capacity In one case there was an increase, suggesting that the adrenal was not secreting maximally prior to ACTH.

5. No conjugated corticosteroids were found in the adrenal venous blood

6. Somewhat more epinephrine than nor epinephrine was secreted by the human adrenal under the circumstances of the investigation The average values were 0.337 μg/minute for epinephrine and 0 115 μg/minute for nor epinephrine.

7. There is a greater urinary excretion of nor epinephrine than epinephrine following surgery in the human, most of the former coming from extra adrenal sources.

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RELATIONSHIP BETWEEN STEROID OUTPUT IN ADRENAL VEIN BLOOD AND STEROID FORMATION OF ADRENAL INCUBATES OR HYPERTENSIVE PATIENTS *

DAVID Y. COOPER, JOSEPH C. TOUCHSTONE, WILLIAM S. BLAKEMORE, OTTO ROSENTHAL AND MARIA KASPAROW

Previous studies from this laboratory[1] have indicated that the rate of cortisol formation by incubated adrenal tissue from patients with severe hypertension (diastolic blood pressure 100 to 120) is elevated when compared with production by incubated tissue from patients with normal blood pressures. As diastolic blood pressure increased, the rate of cortisol formation of the incubates decreased to a level not significantly different from patients without hypertension. The output of corticosterone (Compound B) by the incubates was significantly elevated in all patients with hypertension when compared with control patients.

This report compares the steroid content of samples of adrenal venous blood and incubated adrenal tissue which were obtained simultaneously from ten patients with severe hypertension. Also, this study correlates these findings with the clinical severity of hypertension.

METHOD

1. Patients. Ten patients with severe hypertension (B.P. 180/110 to 245/150), who had been maintained on medical treatment for their hyper-ension and had failed to respond were studied. Nine of these subjects had ight total adrenalectomies at varying intervals of 2 weeks to 3 months pre-ious to the present study. The nine second stage adrenalectomy patients ceived 50 mg. of cortisone intramuscularly at 10 p.m. the night prior to the operation and intramuscularly 4 hours before operation. The patient (McL) undergoing the first stage procedure received no steroid.

2. Adrenal Venous Blood. Adrenal venous blood was obtained prior to resection of the adrenal gland by placing a polyethylene cannula in the adrenal vein after ligation distally and allowing the blood to flow into a heparinized syringe. In 6 patients the collection period was timed for estimation of adrenal blood flow. Values for adrenal vein blood flow ranged from 1.6 to 3.6 cc./min. (See Table 1).

3. Preparation of Extracts of Adrenal Venous Blood and Adrenal Tissue Incubates. The extraction, chromatographic, and quantitative techniques employed in this study have been described in detail in previous papers[2,3] from this laboratory. The blood samples were extracted by the same procedure used for the incubates.

Five definite areas of ultraviolet absorption have been found on the chromatograms of extracts of adrenal vein blood which are similar to those obtained from extracts of incubates tissue slices.[2,3] These have been labeled "before F," F, "E-region," "B-region," and Δ^4-androstene 11-βol-3, 17-dione (11-β-OH).

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania and the Hypertension Section of the B. Robinette Foundation of the Hospital of the University of Pennsylvania, Philadelphia.

Table 1 Adrenal Venous Blood Steroids
(µg/100 cc)

TIENT	B P MM HG	CC OF BLD COL	AD BLD FLOW CC/MIN	BEFORE F	F	E REGION	B REGION TOTAL	BEFORE B	B	S	11 β OH	F/B	F/B REGION
B	210/112	20 0	—	459	1708	249	1038	197	380	210	375	4.50	1 65
W W	220/115	132 0	3 6	228	1168	300	410	118	200	60	246	5 80	2 80
M C	180/120	3.5	—	124	772	255	568	—	—	—	109	—	1 36
H G	190/120	1 8	1 8	172	1243	123	317	58	157	39	98	7 9	3 60
	200/125	28 0	2 4	239	828	102	277	32	92	22	228	9 0	3 00
	230/130	50 0	—	71	510	102	474	—	—	—	160	—	1 00
	220/130	12.5	1 7	404	1102	153	1048	42	490	160	—	2 26	1 06
	210/140	59 0	1 6	114	644	246	616	78	298	76	205	2 16	1 04
R D	220/145	56 0	2 2	189	570	378	646	136	278	73	328	2 05	88
F McL †	245/150	8 0	—	453	1045	739	1454	170	625	00	274	1 67	72

† First stage operation—no cortisone supplement

The "B region" has been found to contain three definite substances absorbing ultraviolet light of 215 mμ when rechromatographed in the methylcyclohexane-dimethyl formamide system.[4] The first zone has been labeled "before B," the second is corticosterone and the last has been identified as Substance S (17 hydroxydesoxycorticosterone).[4]

The amount of steroid (μg/100 cc) in each region of the chromatogram for the 10 patients of this study is tabulated in Table 1.

Steroid Output/100 ml of Adrenal Vein Blood and the Output/Gram/24 Hours by Incubated Adrenal Tissue Figure 1 compares the per cent of total steroid output represented by each of the five regions of ultraviolet absorption in extracts of adrenal vein blood and incubated adrenal tissue. The pattern of steroid output from these two sources appears qualitatively similar in each of the five areas with the possible exception that the incubates tend to form a smaller percentage of cortisol and a slightly larger percentage of 11 β OH.

To determine whether the quantity of steroid in adrenal vein blood was related to the output by incubated adrenal tissue slices, the cortisol and B region steroids were plotted against the content of these same substances in 100 cc of adrenal vein blood as shown in Figures 2 and 3 respectively. Although too few observations are available for statistical comparison, a relationship appears to exist between adrenal vein blood content and steroid output/gm /24 hours by adrenal tissue slices.

Relationship of Total Output/24 Hours to Clinical Hypertension To determine whether the rate of cortisol formation per 24 hours was negatively related to diastolic blood pressure as indicated by the incubation studies,[2] the cortisol output calculated from blood flow (mg /24 hrs) was plotted against the diastolic blood pressure as illustrated in Figure 4. Cortisol output per 24 hours decreased as diastolic blood pressure increased in a manner similar to that observed for incubated adrenal tissue. No relationship of corticosterone output per 24 hours to diastolic blood pressure was found. Likewise the output per 24 hours of S and 11 β OH were not related to diastolic blood pressure. (See Table 1)

F/B Ratio and Diastolic Blood Pressure The lowest F/B ratios were found in patients with the highest diastolic blood pressure regardless of whether the "F to B region" or F to purified B ratios were compared. This suggests a relationship of the F/B ratio to increasing severity of hypertension similar to that reported for the incubation studies.[2] The higher F to B ratios were due to the further purification of the "B region" because of the removal of the "before

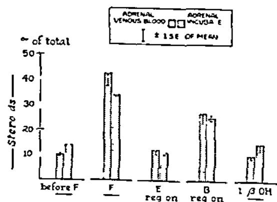


Fig 1 Comparison of the per cent of total steroid output by each of the five regions of ultraviolet absorption. Incubates tend to form slightly smaller percentage of cortisol and slightly larger percentage of 11 β OH.

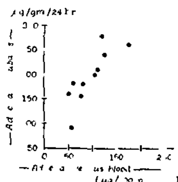


Fig 2 Relationship of cortisol output by incubated adrenal tissue and cortisol content/100 cc of adrenal vein blood

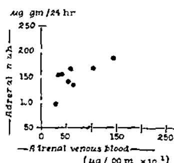


Fig 3 Relationship of B region steroid output by incubated adrenal tissue/24 hours and the B region content/100 ml of adrenal vein blood

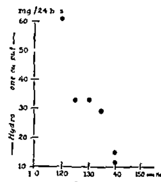


Fig 4 Relationship of the daily cortisol output (μg/24 hours) in adrenal vein blood and diastolic blood pressure The negative relationship is similar to that observed for incubated adrenal tissue

B material and Substance S and of steroid losses from elution and rechromatography

DISCUSSION

The decrease in daily cortisol output (mg /24 hrs) in adrenal vein blood associated with increasing diastolic blood pressure is in agreement with our observations based on incubated adrenal tissue from hypertensive patients[2 3] In this study however we are unable to determine whether corticosterone levels in adrenal vein blood from patients with hypertension are elevated since we have not obtained samples of adrenal venous blood from patients without hypertension The low F/B ratio found in severe hypertensive patients is similar to that observed in the incubation studies although too few observations are available at this time for statistical confirmation of this relationship

From these studies it appears that a definite relationship exists between cortisol and B region output per 24 hours by incubated adrenal tissue and the total daily output of these steroids based on adrenal blood flow and steroid content In view of the similarity of these studies the use of adrenal tissue incubated in autologus plasma *in vitro* as an index of potential capacity of the adrenal to form steroids *in vivo* appears justifiable

SUMMARY

1 A comparison has been made between the steroid formation by incubated adrenal tissue and steroid output of adrenal vein blood in patients with hypertension

2 Under both conditions the cortisol output decreases with the increasing severity of the hypertension as indicated by the diastolic blood pressure

3 Corticosterone output does not change significantly as diastolic blood pressure increases

4 Changes in rate of synthesis of F and B result in a low F/B ratio in severe hypertensives indicating a qualitative change in the pattern of steroid formation in hypertension

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THE USE OF INTRAVENOUS HYDROCORTISONE IN HEMORRHAGIC SHOCK *

JOHN E CONNOLLY DAVID I BRUNS AND RAYMOND C STOFER

The availability of cortisone and other adrenal cortical compounds has markedly affected the care of patients with adrenal cortical insufficiency The effect of cortisone administration to such patients has served to demonstrate the well known relationship of blood pressure to the adrenal cortex It was only natural that coincident with the introduction of cortisone investigators would explore its effect on shock occurring in situations where no obvious adrenal cortical deficiency was evident

Howard and DeBakey[1] reported experiments in which they tested the effects of cortisone on hemorrhagic shock in the dog Under the conditions of their experiments they concluded that cortisone had no significant effect on the course of hemorrhagic shock or on the survival rate of the animals In their report of 1951 they employed 50 to 200 mg of cortisone (Cortone) intramuscularly before operation during the shock period and after retransfusion As one will see from our protocol the time of administration and the size of their dose of the drug differed from ours as well as the route of administration

In 1957 Knapp and Howard[2] again reported the failure of hydrocortisone in hemorrhagic shock The hydrocortisone was administered intravenously in a dose of 100 mg several hours after the hypotensive state had been reached and just before retransfusion was begun Our results are in agreement with theirs namely that if the drug is administered after the period of irreversibility has been reached no beneficial effect will accrue

Frank, *et al*[3] have likewise reported failure of cortisone to increase the survival rate of animals subjected to hemorrhagic shock They employed

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intravenous doses of 15 to 100 mg of cortisone given (1) prophylactically (2) at the end of the shock period or (3) after the replacement transfusion They did not report any experiments in which large doses of hydrocortisone were administered early in the period after severe shock had been induced

In spite of these reports intravenous hydrocortisone is fairly widely employed empirically in cases of surgical shock which have not responded to transfusions of blood or to vasopressors even though true adrenal insufficiency is not evident There are numerous reports in the literature where the administration of hydrocortisone in such instances has appeared to be lifesaving[4 5 6] The personal observation of the response to hydrocortisone in several patients in shock prompted us to reinvestigate its value in the research laboratory

Our initial efforts have been to study the effects of intravenous hydrocortisone † on hemorrhagic shock

Case Histories

1 P L (*History furnished by Doctor E J Harris San Mateo California*) The patient suddenly collapsed on the ward while recuperating from an operation in which a segment of the abdominal aorta was replaced by a homograft No blood pressure or pulse was obtainable and the apex beat was barely palpable 200 mg of hydrocortisone were administered immediately by vein The blood pressure rose to 70/40 mm Hg and remained there for 30 minutes It was further elevated at this time by the administration of norepinephrine to 100/60 mm Hg Two hours later cross matched blood was available and a successful replacement of the ruptured homograft was accomplished The patient recovered uneventfully

2 W Y (History furnished by Doctor L Garlington S U H San Francisco) During an operation for removal of an abdominal aneurism the blood pressure became unobtainable in spite of complete blood replacement and the use of neosynephrine 100 mg of hydrocortisone were given intravenously Three minutes later the blood pressure had risen to 110/65 It gradually rose to 130/80 mm Hg and remained there for four and one half hours at which time the operation was completed Six hours later it was necessary to perform an embolectomy of the left femoral artery The blood pressure again became unobtainable 100 mg of hydrocortisone were again given and a level of 100/60 mm Hg was reached and maintained for the remainder of the procedure

METHOD

The standard shock preparation of Wiggers was adopted[7] Mongrel dogs were anesthetized with barbiturates Catheters were placed in both femoral arteries, one for bleeding the animal and the other for blood pressure monitoring through a mercury manometer A catheter was placed in one femoral vein for administration of blood or drugs The animals were bled into plastic bags containing 120 ml of ACD solution B Bleeding was carried out rapidly until a mean pressure of 50 mm Hg was reached This pressure was maintained for 90 minutes by occasional small withdrawals of blood The amount of bleeding required to reach 50 mm Hg was roughly 50 ml /kg After the 90 minute period further blood was withdrawn to reduce the blood pressure to 30 mm Hg This pressure was maintained for 45 minutes Small infusions were given if the pressure tended to decline toward the end of this period At

† The hydrocortisone used was Solu Cortef supplied by Dr Hubert C Peltier of The Upjohn Company

the end of this time the entire amount of blood that had been withdrawn was reinfused Wiggers reported an 82% mortality within 6 hours after reinfusion Twelve control dogs were bled in this manner Thirty seven additional dogs were bled according to the above protocol but to test the effects of our drug 100 to 300 mg of hydrocortisone (Cortef) dissolved in 2 to 4 ml of saline were given intravenously at various times during the bleeding cycle If the administration of hydrocorticone elevated the blood pressure the length of time that the elevation was maintained was recorded

RESULTS

Of the 12 control dogs 11 died of irreversible shock within 12 hours Table 1 shows the results of the 37 dogs administered hydrocortisone Except in 2 dogs the drug was uniformly successful in elevating the blood pressure to safe levels (90 to 100 mm Hg) when administered within 30 minutes after a mean pres sure of 40 to 50 mm Hg had been reached Occasionally the hydrocortisone was successful in elevating the blood pressure as long as 15 minutes after a pressure of 40 to 50 mm Hg had been reached But the drug had no effect after this 45 minute period The severity of this shock preparation is attested by the death of 13 dogs not included above whose blood pressure suddenly fell to fatal levels while attempting to maintain the 30 minute period of shock The average amount of drug required was 200 mg Occasionally 100 mg were successful in producing a blood pressure response and more often 300 mg were required If 300 mg were unsuccessful in effecting a response larger amounts of hydrocortisone were of no avail If the hydrocortisone was success ful its effect was almost always seen within 5 minutes after its administration and never later than 10 minutes When the drug was administered early and was successful in elevating the blood pressure to figures over 80 mm Hg the effect of the single injection usually extended over an average period of 3 hours Figure 1 shows a diagram of a typical experiment in which a dog was rapidly bled to a blood pressure of 40 mm Hg and held there for 30 minutes at which time 300 mg of hydrocortisone were given intravenously Within a few minutes the blood pressure rose to 115 to 120 mm Hg and remained there for a period of several hours

DISCUSSION

From our experiments it is apparent that once hemorrhagic hypotension below 50 mm Hg has been allowed to exist uncorrected longer than 45 min utes a state of irreversibility as far as hydrocortisone s effect is concerned

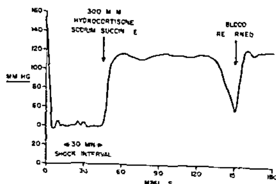


Fig 1 The changes in blood pressure dur ing a typical experiment are shown

Table 1. Hemorrhagic Shock

SHOCK LEVEL	DURATION OF SHOCK	NUMBER OF ANIMALS	SATISFACTORY RESPONSE TO INTRAVENOUS HYDROCORTISONE		DURATION OF RESPONSE
	MIN		NUMBER	AVER. SYSTOLIC BP MM. HG	MIN.
30 mm Hg	5	3	3	90	120 to 150
	15	2	1	95	120
	25	1	0	—	—
	45	2	1	100	100
40 mm Hg	5	1	1	105	135
	20	3	3	95	60 to 300
	25	1	1	80	120
	30	7	6	90	30 to 110
	45	2	0	—	—
50 mm. Hg	5	1	1	100	135
	15	2	2	100	15 to 270
	30	7	5	100	45 to 420
	45	3	1	90	90
	90	2	1	80	15
		37	26		

has been reached. Up to 30 minutes after profound hypotension due to hemorrhage has been reached, hydrocortisone given in large doses intravenously will effect spectacular elevations in the blood pressure and maintain this elevation for long periods of time without blood replacement or other treatment. Since its action depends so precisely on how long the hypotensive state has been present, the intravenous route has been mandatory when dealing with critical levels of hypotension such as produced in our experiments. If hydrocortisone is given orally or intramuscularly, a period of more than 30 minutes is required before maximum blood levels are obtained.[5]

The mechanism by which hydrocortisone and its related compounds elevate the blood pressure and prevent so-called irreversible shock is not definitely known. Zweifach [8] and Fritz and Levine [9] have demonstrated that in adrenalectomized animals vascular response to nor-epinephrine is lost and is restored only by the topical application of adrenal extracts or by the injection of cortisone. Hayes [10] states that in hemorrhagic shock there is a decreased minute volume of blood delivered to the kidneys and presumably to the adrenal glands, with a subsequent possible reduction in the amounts of adrenal cortical steroids available in the circulation per unit time for peripheral tissue utilization. On the other hand, the work of Walker [11] indicates that this is not so and that the steroid output of the adrenals is not affected by hypotension. In view of the massive doses of hydrocortisone required, it is quite likely that the levels of circulating corticosteroids which can be produced by the adrenals in response to shock is inadequate to maintain a satisfactory vascular response.

It would appear from our experiments that in the event of hemorrhage severe enough to lower the blood pressure below 80 mm. Hg one should first attempt immediate replacement of the blood lost. However, if blood is not immediately available or the hypotension does not respond to its administration, a large dose of hydrocortisone should be given intravenously within 30 minutes after the hypotensive state has commenced. Our experiments would suggest the comparable adult human dose to be from 500 to 1000 mg. However, a number of clinical cases in which response occurred with 100 mg. have been verified.[4, 5, 6] Therefore the initial dose should be 100 to 200 mg. of hydrocortisone intravenously.

The common practice of giving nor-epinephrine during such emergencies bears further investigation. Close *et al.*,[12] have shown that by giving nor-epinephrine during and after hemorrhagic shock, the mortality rate is twice that of control animals. They conclude that an increase in vasoconstriction is harmful during hemorrhage, "though administration of pressor agents may exert a considerable and beneficial constrictor effect peripherally, they may also overcome the local metabolic factors which normally operate to assure adequate blood flow to the splanchnic bed, liver and to a lesser extent the heart and skeletal muscle."

The simplicity of administering a single injection of an easily carried drug leads us to hope that the development of irreversible shock may be prevented until such a time as blood or intravenous fluids may be given.

SUMMARY

1. An experimental study of the effect of intravenous hydrocortisone on hemorrhagic shock in the dog is described.

2 Hydrocortisone administered in doses of 100 to 300 mg effected spectacular and sustained elevation of the blood pressure of dogs in profound hemorrhagic shock if it was administered within 30 minutes after the state of severe shock was reached It had no effect on restoration of blood pressure if given later than 45 minutes after the severe shock state had been induced

3 A discussion of the possible mechanism of its action in hemorrhagic shock is presented

4 The use of hydrocortisone as an emergency treatment in patients following severe hemorrhage is described

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EFFECT OF HYDRALAZINE ON SURVIVAL OF DOGS SUBJECTED TO HEMORRHAGIC SHOCK *

Preliminary Report

WALTER ZINGG MARK NICKERSON AND STEFAN A CARTER

Previous studies have demonstrated convincingly that pretreatment with vasodilators usually adrenergic or ganglionic blocking agents may increase the percentage of animals surviving various procedures designed to induce fatal shock[1] However, interpretation of these data is difficult Pretreated animals may reach standard levels of hypotension with less blood loss than controls and thus the improved survival rate might be attributable to decreased stress rather than to increased resistance The present experiments were designed to isolate and study the effects of the vasodilator hydralazine (Apresoline®) on resistance to a hemorrhagic stress

* Departments of Surgery and of Pharmacology and Therapeutics University of Manitoba Faculty of Medicine Winnipeg Manitoba Supported by grants in aid from the Defence Research Board of Canada and Ciba Company Limited

Paired dogs were simultaneously subjected to a modified Wiggers constant pressure procedure[2] for the induction of hemorrhagic shock Under light pentobarbital anesthesia one brachial and one femoral artery were cannulated with polyethylene tubing the former for determining arterial pressures and the latter for bleeding Pressures were measured with mercury and water manometers the latter whenever the pressure was below 70 mm Hg After intravenous administration of 5 mg/kg of heparin the animals were bled into a closed reservoir system in which the pressure was maintained constant by a slow flow of oxygen under controlled pressure Bleeding was manually controlled to reduce the mean arterial pressure to 45 mm Hg over a period of 10 minutes After an additional 30 minutes at this level the pressure was raised to 70 mm Hg for 30 minutes Some reinfusion of blood occurred immediately after the upward adjustment of the pressure but the animals then continued to bleed out slowly and the bleeding volume at the end of the 70 mm Hg period usually was equal to or greater than that at the end of the 45 mm Hg period After equilibration at the 70 mm Hg pressure the tubing connecting the femoral artery to the reservoir was clamped for 90 or 120 minutes The period was always the same for both members of a pair but was increased to 120 minutes during one period during the course of the experiments because of an increased average resistance of the animals used All of the withdrawn blood was then reinfused over a 10 minute period and the animals were followed for 18 hours to evaluate survival Hydralazine (0 5 mg/kg) was administered intravenously to one animal of each pair selected by random numbers immediately after the tubing to the blood reservoir was clamped Bleeding volume blood pressure heart rate and respiratory rate were recorded at 5 minute intervals throughout the experiment

Figure 1 illustrates the results of a representative experiment The blood pressure of the hydralazine treated dog invariably fell earlier in the clamped period than did that of the control animal but the fall usually persisted for less than one hour and the pressure then stabilized or rose slowly during the remainder of the period In contrast the blood pressure fall in control animals once started was always progressive Analysis of the results obtained from 16 pairs of animals showed that the control and experimental groups did not differ significantly in weight amount of anesthetic required initial blood pressure or initial maximal or final bleeding volumes

Two important differences between the experimental and control groups were demonstrated (Table 1) The percentage survival in the treated group was considerably higher Of the 16 pairs studied 11 hydralazine treated and 5 control animals survived a highly significant difference ($P < 0\ 01$) In addition the average duration and severity of hypotension compatible with survival was considerably greater in the treated than in the untreated group

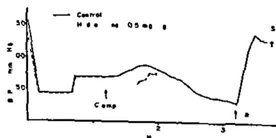


Fig 1 Arterial pressure records of a representative pair of dogs during the total experimental period Hydralazine was administered to one animal immediately after the tubing to the blood reservoir was clamped

Table 1 Effects of Hydralazine on Response to Hemorrhagic Shock

GROUP		NO	% SURVIVAL	HYPOTENSION (MIN X MM <70)* MAX SURVIVAL OR MIN LETHAL	AVERAGE
Control	Survived	5	31	1160	32
	Died	11		350	991
Hydralazine (0.5 mg/kg)	Survived	11	69	1785	963
	Died	5		1340	1940

* The figures given are the algebraic sum of the magnitude and duration of blood pressure variations above and below 70 mm Hg during the "clamped" period calculated from determinations at 5 minute intervals

With only one exception the control animals which survived were those whose mean arterial pressure remained at 70 mm Hg or above during the entire "clamped" period The average hypotension experienced by survivors in the treated group was comparable to that of the controls which died and *in the treated group fatalities occurred only among those animals which* were severely hypotensive during most of the "clamped" period

CONCLUSIONS

On the basis of the above observations it may be concluded that when administered during a procedure leading to hemorrhagic shock and precluding drug induced alterations in bleeding volume hydralazine can provide significant protection against the development of a condition irreversible to reinfusion It also is apparent that animals treated with a vasodilator such as hydralazine can survive a much more prolonged and severe hypotension than can controls studied under identical conditions

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THE EFFECT OF HEMORRHAGIC SHOCK ON INTESTINAL BLOOD FLOW*

WALTER S. HENLY, ROBERT C. OVERTON, JAMES D. McMURREY, KENNETH M. GRIFFITH, AND MICHAEL E. DEBAKEY

Previous studies of plasma volume, red cell volume and blood volume before, during and after hemorrhagic shock have demonstrated a loss to the effective circulation of a considerable quantity of returned blood, particularly plasma.[1] These findings suggested as possibilities for this discrepancy the presence of an area where trapping of red blood cells and plasma occurred, an area where the mixing time was very much prolonged, and/or an area where damage to the capillary bed might promote the loss of blood from the circulation after replacement by transfusion. The demonstrations of a protective effect in hemorrhagic shock with oxygenation and preservation of blood flow to the gastrointestinal tract by cross circulation[2] and the less striking but beneficial effect of lower temperature of the gastrointestinal tract during hypothermia[3] strongly point to the gastrointestinal tract as a site of "irreversibility" in hemorrhagic shock. It seemed desirable, therefore, to measure objectively changes occurring in blood flow to the gastrointestinal tract before, during and after massive blood loss had occurred.

METHOD

A standardized shock preparation, Wiggers graduated hemorrhage, was utilized in twelve anesthetized mongrel dogs. Blood pressures were measured with a mercury manometer connected to a catheter inserted in a femoral artery with a T tube connection to a Lampson bottle for collection of blood during hemorrhage. The opposite femoral vessels were cannulated for constant injection of RISA via the vein and for withdrawal of continuous blood samples from the artery. The superior mesenteric artery and the portal vein were also cannulated, the artery for injection of the RISA, and the vein for withdrawal of samples. Perforated tip cannulae which did not occlude flow in any of the vessels were utilized. Infusion of the isotope was maintained at a constant known rate by a mechanically driven calibrated syringe. The withdrawal of blood for sampling was at a controlled rate utilizing a vacuum system. The withdrawal sample was circulated immediately through a scintillation counter with the crystal modified to allow the catheter to pass directly through it. Radioactivity was measured by an analytical count rate meter and recorded graphically. Cardiac output and portal blood flow were calculated from the radiodilution curve. These measurements were made before, during and after production of hemorrhagic shock (Fig. 1).

RESULTS

The individual values for the twelve dogs including weight, blood pressure, cardiac output, portal blood flow, and portal blood flow calculated as a percentage of cardiac output during the various phases of the hemorrhage

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* From the Cora and Webb Mading Department of Surgery, Baylor University College of Medicine. Supported in part by the Department of the Army, Research and Development Division, through Contract No. DA 49 007 MD 564, and grants from the American Heart Association and the Texas Heart Research Foundation.

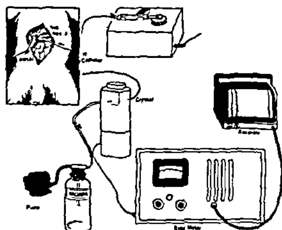


Fig 1 Apparatus for determination of portal blood flow

are listed in Table 1 The mean portal blood flow at normotensive levels was 496 cc/min, or 31 6 cc/min/kg, and represented 28 1% of the cardiac output At hypotensive levels of blood pressure, the mean portal blood flow was 272 cc/min, or 18 5 cc/min/kg, which represented 25 5% of the cardiac output The portal blood flow was thus reduced by a factor of 38 4% Additional measurements of portal blood flow in two dogs in which normotensive levels of blood pressure were obtained following retransfusion of the shed blood demonstrated a 37% increase in portal flow over that obtained in the preshock period In some animals a measurement of portal blood flow was obtained while the animals were actively bleeding at hypotensive levels and

Table 1 Changes in Portal Blood Flow in Hemorrhagic Shock

	NORMOTENSIVE					HYPOTENSIVE			
WEIGHT (KG)	B P (MM/HG)	C O (L/MIN)	PORTAL BLOOD FLOW (CC/MIN)	PORTAL BLOOD FLOW (CC/MIN/KG)	PER CENT C O	B P (MM/HG)	PORTAL BLOOD FLOW (CC/MIN)	PER CENT C O	CHANGE IN PORTAL BLOOD FLOW (%)
12 0	110	2 12	630	52.5	29 7	48	422	—	33 6
14 0	80	0 90	253	18 1	28 1	15	230	—	9 1
20 3	135	1.56	445	19 9	28.5	50	323	22.2	27 4
15 0	80	1 73	331	22 1	19.2	40	232	25 1	29 9
10 4	90	1 45	465	44 7	32 1	40	135	8 4	71 0
13 2	80	1 40	461	34 9	32 9	30	275	—	40.3
18 0	110	1 73	555	30 8	32 1	35	326	26 7	41 3
15 6	110	1 38	460	29.5	33 3	35	246	28 4	46.5
14 4	120	2 06	580	40.2	28.2	20	418	—	27 9
13 6	115	1 34	436	32 1	32 4	20	185	32 6	57 5
13.2	115	1 76	461	34 9	26.2	30	227	—	50 8
17 0	125	1 38	341	20 0	24 7	50	255	35 1	24 6
14 7			*496*	*31.6*	*28 1*		*272*	*25.5*	*38 4*

again after additional blood had been lost but hypotension was stabilized without bleeding. In these dogs the blood flow through the gastrointestinal tract decreased by a factor of 49.2% below the normotensive portal blood flow.

DISCUSSION

The method utilized in this determination of blood flow utilizes a short period of injection of the radioisotope at a constant rate and a continuous, direct recording of radioactivity in sampled blood. This method has proved to have a minimum error when used in a mechanical flow system, and for the same mechanical system the error is reduced to less than 2% with variation in flow rate. The values for portal blood flow agree in general with those of other investigators.[4] Portal blood flow in all animals showed an absolute decrease in hemorrhagic shock. The average portal blood flow in the normotensive and hypotensive states was comparable when calculated as a percentage of the cardiac output in the two conditions. However, the individual values for the various dogs showed fluctuation above and below this mean. It is possible that the portal blood flow in the hypotensive animals was reduced to a level which was not sufficient to maintain normal viability of the gut.

In a few animals with blood pressure unchanged and at hypotensive levels, the portal blood flow was strikingly reduced during active bleeding below that obtained in the stabilized hypotensive state. Insofar as the blood flow to the gastrointestinal tract is concerned, it is apparently more deleterious to have continued blood loss taking place than to have a greater overall degree of blood loss with the same stable blood pressure. Of further interest is the fact that a more rapid rate of portal blood flow is maintained in animals which have been previously made hypovolemic than is present in the normal animal. This may well reflect a loss of vasomotor tone in the blood vessels of the gastrointestinal tract which persists after correction of the hypovolemia.

This study suggests that an accurate simple method of portal flow determination is now available and that further objective studies in hemorrhagic shock will be possible.

SUMMARY

Determinations of cardiac output and portal blood flow were carried out in twelve dogs before, during, and after production of hemorrhagic shock, utilizing an apparatus for the constant injection of radioactive iodinated human serum albumin with a continuous, directly recording sampling technique. In all animals there was a considerable reduction of portal blood flow after production of hemorrhagic shock. In a few animals in which portal blood flow was measured during actual hemorrhage, the values were considerably below portal blood flows found in the stabilized hypotensive period. An increase in portal blood flow over that present in the normal animal was found following reinfusion and restoration of blood pressure to normotensive levels.

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A METHOD FOR THE MEASUREMENT OF BLOOD FLOW IN ISOLATED SEGMENTS OF SMALL BOWEL AND KIDNEY IN DOGS *

WILLIAM A. NEELY AND M. D. TURNER

The procedure to be described is a method of blood flow measurement through an intact vessel, thus avoiding the disadvantages inherent in any method requiring incision of the vessel and the use of anticoagulants.[1,2]

In addition, the equipment necessary is not so specialized as that used in other methods of measurement of blood flow through an intact vessel.[3,4,5]

METHOD

A pentobarbital anesthetized dog was incised in the midline and the left kidney and its vessels dissected free of all surrounding tissue, including nerves, back to the aorta and inferior vena cava

The other viscera were retracted and the kidney suspended in a nylon bag from a Statham strain gauge transducer (G-1-8 350-), keeping the blood vessels horizontal (Fig. 1).

The transducer was connected to a Sanborn 150 series recorder and the kidney weighed by zero suppression. The sensitivity was then advanced to *x1*, a suitable speed selected, and the vein suddenly occluded. Change in *weight with respect to time was recorded; this change in weight being equal* to blood flow for a very short period of time. The recording was such that a

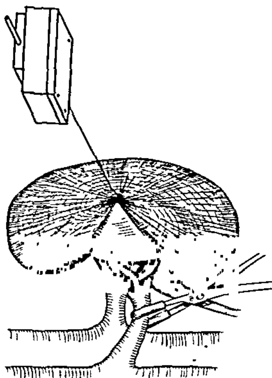


Fig 1. The renal vessels have been dissected free from surrounding tissue The kidney is enclosed loosely in a nylon net, and suspended from a Statham displacement transducer.

* From the Departments of Surgery and Biochemistry of the University of Mississippi Medical Center, Jackson Supported in part by grants from the Mississippi Heart Association, Inc., and from the Office of the Surgeon General, Department of the Army, Contract Number DA-49 007 MD-627.

straight line could be drawn through the first portion and blood flow in cc (synonymous with grams in this study) per minute calculated from the slope of the line. Triplicate determinations were done each time and the average used for statistical analysis

Isolated segments of intestine prepared in a similar fashion were used for measurement of intestinal blood flow.

After determination of renal blood flow by the above described method the renal artery was clamped, (always less than one minute), the renal vein cannulated, and after a short period of free draining, allowed to drain into a beaker placed on an aluminum pan which was suspended from the same transducer. The change in weight of the beaker was recorded as blood flow.

RESULTS

Triplicate Measurements. Statistical analysis reveals no significant difference in the means of three consecutive determinations done on each of 12 gut segments and 18 kidneys

Comparison of Two Methods. In 14 dogs the mean renal blood flow by the weight method was 98 5 cc per minute whereas the mean renal flow by the cannulation method was 79 5 cc. per minute The difference was not significant ($P > 05$) The individual determinations by the weight method correlated well ($r = 80$) with the corresponding determinations of direct cannulation.

DISCUSSION

Table I shows that the results of this method are reproducible.

Table I

	MEAN BLOOD FLOWS 1	2	3	STANDARD ERRORS OF MEANS 1	2	3
Gut Segments	0.29 †	0.27	0.27	0 042	0 039	0 035
Kidney	1.53	1.59	1 63	0 17	0 17	0 17

† All figures in cc /minute/gram of tissue

Comparison of the results of the weight method and the cannulation method show a mean flow which is less as measured by the latter method, and is insignificant in this small series, but might be significant in a larger series If so, this is probably due to resistance introduced by the cannula, thus the weight method is as reliable as the other, and it is certainly more simple

In using the weight method certain precautions must be observed The vessels must be kept horizontal to eliminate the effect of lengthening of the vein under high pressure upon the weight of the segment The clamps used to occlude the vein should be rubberized The clamp must be held at right angles to the horizontal vein in order to eliminate the "Bourdon" effect

SUMMARY

1 A simple method of measuring blood flow in kidneys or intestinal segments is described.

A METHOD FOR THE MEASUREMENT OF BLOOD FLOW IN ISOLATED SEGMENTS OF SMALL BOWEL AND KIDNEY IN DOGS *

William A Neely and M D Turner

The procedure to be described is a method of blood flow measurement through an intact vessel thus avoiding the disadvantages inherent in any method requiring incision of the vessel and the use of anticoagulants [1 2]

In addition the equipment necessary is not so specialized as that used in other methods of measurement of blood flow through an intact vessel [3 4 5]

METHOD

A pentobarbital anesthetized dog was incised in the midline and the left kidney and its vessels dissected free of all surrounding tissue including nerves back to the aorta and inferior vena cava

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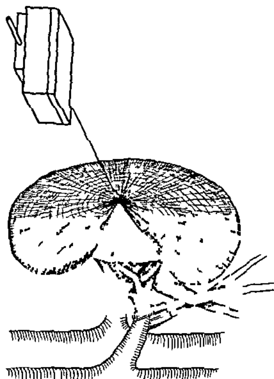


Fig 1 The renal vessels have been dissected free from surrounding tissue The kidney is enclosed loosely in a nylon net and suspended from a Statham displacement transducer

* From the Departments of Surgery and Biochemistry of the University of Mississippi Medical Center Jackson Supported in part by grants from the Mississippi Heart Association Inc and from the Office of the Surgeon General Department of the Army Contract Number DA 49 007 MD 627

straight line could be drawn through the first portion and blood flow in cc (synonymous with grams in this study) per minute calculated from the slope of the line. Triplicate determinations were done each time and the average used for statistical analysis.

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After determination of renal blood flow by the above described method the renal artery was clamped (always less than one minute), the renal vein cannulated and after a short period of free draining allowed to drain into a beaker placed on an aluminum pan which was suspended from the same transducer. The change in weight of the beaker was recorded as blood flow.

RESULTS

Triplicate Measurements. Statistical analysis reveals no significant difference in the means of three consecutive determinations done on each of 12 gut segments and 18 kidneys.

Comparison of Two Methods. In 14 dogs the mean renal blood flow by the weight method was 98.5 cc. per minute whereas the mean renal flow by the cannulation method was 79.5 cc. per minute. The difference was not significant ($P > .05$). The individual determinations by the weight method correlated well ($r = .80$) with the corresponding determinations of direct cannulation.

DISCUSSION

Table 1 shows that the results of this method are reproducible.

Table 1

	MEAN BLOOD FLOWS			STANDARD ERRORS OF MEANS		
	1	2	3	1	2	3
Gut Segments	0.29†	0.27	0.27	0.042	0.039	0.035
Kidney	1.53	1.59	1.63	0.17	0.17	0.17

† All figures in cc. /minute/gram of tissue

Comparison of the results of the weight method and the cannulation method show a mean flow which is less as measured by the latter method and is insignificant in this small series but might be significant in a larger series. If so, this is probably due to resistance introduced by the cannula; thus the weight method is as reliable as the other and it is certainly more simple.

In using the weight method certain precautions must be observed. The vessels must be kept horizontal to eliminate the effect of lengthening of the vein under high pressure upon the weight of the segment. The clamps used to occlude the vein should be rubberized. The clamp must be held at right angles to the horizontal vein in order to eliminate the "Bourdon" effect.

SUMMARY

1. A simple method of measuring blood flow in kidneys or intestinal segments is described.

2 The reproducibility of the method is good as shown by triplicate determinations

3 There was no significant difference between the results obtained by this method and the results obtained by the cannulation method of measuring blood flow

4 Subsequent determinations of blood flow by the cannulation method after determination by the weight method correlated well

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HEPATIC BLOOD FLOW IN HEMORRHAGIC SHOCK *

WILLIAM C SHOEMAKER WILLIAM F WALKER
AND FRANCIS D MOORE

METHOD

A simplified experimental preparation has been devised in this laboratory to study hepatic blood flow and regional hepatic metabolism in the unanesthetized dog [1] Taking advantage of an anatomical relationship peculiar to the dog we have found it possible to gain access to the left common hepatic vein through the midline upper abdominal approach A catheter is placed in the left common hepatic vein at the confluence of the veins draining the left central and left lateral lobes the position of the tip of the catheter is approximately 4 to 5 cm from the junction of the left common hepatic vein with the vena cava After splenectomy the portal vein is catheterized and the splenic artery cannulated All three catheters are brought out through a stab wound in the left flank Following recovery from the operation repeated blood samples may be obtained from these catheters under basal or resting conditions without anesthesia

Cardiac output was estimated by the direct Fick oxygen method and oxygen consumption by a Benedict Roth spirometer using an endotracheal tube placed through a previously devised tracheotomy orifice Arterial blood pressure was measured with a dampened mercury column

Hepatic blood flow (HBF) was measured using modifications of the Bromsulphalein (BSP) method of Bradley *et al* [2] The modified BSP method [3] has been compared with regional hepatic blood flow measurements by a method

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independent of hepatocellular function using the Cr^{51} labeled red cells When the conditions and assumptions of these methods were fulfilled a mean deviation of approximately 6% was found [7]

Ten dogs subjected to hemorrhagic shock were compared in 5 dogs 200 to 100 ml of blood were suddenly withdrawn in 5 dogs 10 to 20 cc. were withdrawn every 10 minutes until death

RESULTS

A Acute Hemorrhage Following the sudden withdrawal of 200 to 100 cc of blood representing 1/6 to 1/4 of the calculated blood volume a pronounced fall in blood pressure, cardiac output oxygen consumption pulmonary artery and hepatic venous oxygen content was regularly observed A rise in peripheral resistance systemic (arterial right ventricle) and splanchnic (arterial portal) oxygen gradients was similarly noted Table I summarizes the data of one of the dogs With this degree of hemorrhage a decrease in hepatic blood flow was noted in three of the five animals In the two remaining dogs the hepatic blood flow was found to be essentially unchanged despite changes in blood pressure and cardiac output

Table 1

Dog 3 4 10-57
19 kg
Hemorrhage 300 cc

	BASELINE	AFTER HEMORRHAGE
Blood Pressure	120 mm Hg	60 mm Hg
O_2 Consumption	109 cc/min	81
Cardiac output	4.2 L/min	1.4
Peripheral Resistance	286 units	430
Hepatic Blood Flow	382 ml/min	404
Systemic O_2 gradient (Art – Rt Vent O_2 Conc)	2.58 Vol %	5.79 Vol %
Splanchnic O_2 gradient (Art – H V O_2 Conc)	4.28	10.98
(Portal V – Hepatic V)O_2 Gradient	2.05	0.37

B HBF in Continuous, Prolonged Hemorrhage. During slow continuous hemorrhage in 5 dogs HBF was measured at 10 minute intervals until death occurred in 4 to 12 hours Data from a representative experiment are summarized in Table 2 In the initial period flow values were stable This period was found to extend until approximately 10 to 20% of the measured or calculated blood volume was withdrawn Following this there was a period of erratic increases in hepatic blood flow This increase in flow was not accompanied by obvious excitement or agitation In the animals studied this increase was 45 to 300% over the control value This period of increased flow

was brief but definite The third and longest period was characterized by a gradual but continually decreasing HBF Toward the end of this period there was diminished clearance of BSP This is first manifest by a slight increase of portal vein BSP levels The fourth and final period was characterized by a further decrease in the rate of HBF and a progressively decreasing functional capacity of the liver as evidenced by a decreasing ability of the liver to clear BSP This progressive impairment of hepatic function makes HBF calculations less reliable in spite of correction factors

Table 2

Dog 19 10-25-57
16 2 kg
Blood Volume—1550 ml
Total Amount of Bleed—800 ml
Total Time of Hemorrhage—4½ hours

STAGE	NUMBER OF DETERMINATIONS	PERIOD OF TIME	HPF	HBF
1 Control	4	50 min	460 ± 54 ml/min	690 ml/min
2 Increased Flow (Overcompensation)	4	50 min	1350 ± 600	1985
3 Decreased Flow	12	120 min	300 ± 89	428
4 Functional Decompensation	4	50 min	215 ± 37	275

DISCUSSION

The findings in this preliminary investigation suggest that after a sudden acute hemorrhage the hepatic blood flow tends to be preferentially maintained The second portion of this study was specifically designed to describe and characterize the early changes in HBF during progressive hemorrhage Frequent measurements of HBF during slow but continuous blood withdrawal allow almost continuous evaluation of the status of hepatic flow in the early stages of unanesthetized hemorrhage In the experiments described just enough blood for the BSP analyses (5 to 10 cc) was aspirated from the portal and hepatic venous catheters at 10 minute intervals Under these experimental circumstances four stages were found (1) Initial stage wherein the baseline values were maintained (2) A period of increased flow thought to be a compensatory reaction possibly mediated by an increased production of catechol amines or neural influences (3) A prolonged period of progressively decreasing flow culminating in (4) a final stage of hepatic functional decompensation with progressively decreasing ability of the liver to clear BSP

Hemorrhage or hemorrhagic shock has been most frequently studied after an acute bleeding or use of the Lampson bottle (or Wiggers) technique With these methods a marked decrease in HBF has been reported [4, 5] The advantage of the Wiggers method is that a relatively stable hemorrhaged

preparation is provided for a period of several hours. This preparation is reproducible from laboratory to laboratory and has become the standard or classic technique for the investigation of shock. One of the disadvantages of the Wiggers method is that the animal is suddenly plunged into late or irreversible shock; this state is then artificially prolonged by the gradual return of the shed blood at a rate sufficient to maintain the blood pressure at a predetermined value. A second disadvantage of the method is that the common point of reference is the mean blood pressure. As the blood pressure represents the sum total of numerous known and unknown compensatory reactions, *it may not be the most ideal referent, especially in the early stages of hemorrhage* Furthermore, the mean blood pressure is only roughly correlated with the percentage of blood volume removed or the rate of the bleed.

In attempting to assess the physiologic reactions to a given stimulus it would seem to be desirable to quantify the bodily reactions to the stimulus rather than to standardize the stimulus and organism to its indirect and compensatory reactions For these reasons, therefore, a model which conforms more closely to the clinical situation was selected, a model wherein the stimulus was held constant and the physical reactions to it were measured as a function of time

SUMMARY

Hepatic blood flow was measured after hemorrhage in unanesthetized dogs whose hepatic vessels were previously catheterized Sudden acute hemorrhage was compared with a gradual, continuous hemorrhage. The advantages and disadvantages of both types of experimental design are briefly outlined.

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BLOOD FLOWS TO VARIOUS TISSUES IN ENDOTOXIN SHOCK *

J BRADLEY AUST AND JOHN A JOHNSON

The administration of E Coli endotoxin into the dog provokes a state of irreversible shock characterized by an immediate decrease in blood pressure due to decreased venous return [1 2] from initial hepatic venous spasm followed by a progressive sequestration of plasma in the intestine,[3] eventually resulting in death A study of the distribution kinetics of tags of plasma, red blood cells, extracellular space, and water space gives information as to the distribution of these tags in the tissues and information concerning the blood flow to these tissues Substances such as D_2O and antipyrine distribute themselves so rapidly across the capillary wall that the factor which limits their rate of distribution is the rate of blood flow to the given tissue This being the case, the principle of conservation of material can be applied to approximate the blood flow to the tissue studied

$$\text{Amount of } D_2O \text{ in} = \text{Amount out} + \text{Amount in Tissue}$$

Flow X Concentration Arterial Blood — Flow X Concentration Venous Blood Leaving Tissue = Amount in Tissue

$$\text{Flow} = \frac{\text{Amount in Tissue}}{\text{(Concentration Arterial Blood Concentration Venous Blood)}}$$

METHOD

The tagged substances, namely RIHSA Cr^{51} tagged red cells, sucrose or thiocyanate and D_2O or antipyrine were injected simultaneously into normal and endotoxin shocked dogs Samples of blood and biopsies of skin muscle intestine, and liver were taken at 2 5, 15 30, 60 and 120 min following injection These samples were then assayed for the amount of their respective tagged substances

The dosage of endotoxin given was one which universally produced irreversible shock Five normal or control studies were compared with five studies following endotoxin administration

The D_2O determinations were carried out using the mass spectrometer technique A vacuum distilled aliquot equilibrated with hydrogen was employed to determine percentage of D_2O Approximately 50 ml of D_2O was injected intravenously per dog The amount of antipyrine injected intravenously at time equal 0 was 400 mg per kg The 0 5 gram samples of tissue for antipyrine determination were extracted with 5 ml of 7 7% zepheran chloride, protein chromogen complexes precipitated with 5 0 ml of 20% trichloro acetic acid and the sample centrifuged for one hour at 2,000 rpm Three milliliters of supernatant were subjected to color development with 2 gtts of fresh 0 4% $NaNO_2$ and read at 350 Mu on a B & L spectrophotometer after maximum color development Standard curves of the various tissues gave linear plots with little evidence of tissue blank interference

The assumption of conservation for D_2O and antipyrine distribution in

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the first 2 min leads to the following calculation. The flow is estimated by taking the amount of tag in the tissues at the end of 2 min, divided by the difference between arterial concentration in the first min. (i.e. the average D_2O for the first 2 min) and one half the tissue value (as an approximation of the venous concentration leaving the tissues)

$$\text{Flow} = \frac{\text{Amount Tissue}}{(\text{Arterial concentration} - 1/2 \text{ tissue concentration})}$$

RESULTS

Figure 1 shows the kinetics of distribution of D_2O in the normal animal revealing that equilibrium in the tissues was established by 2 hours. The levels of D_2O in liver and intestine actually crossed the percentage in the blood in the first 2 min. following injection, indicative of a rapid flow rate and equilibration of these tissues with D_2O in the blood. They then followed the blood curve down as other tissues such as muscle and skin, which equilibrated more slowly, picked up the tagged water.

Figure 2 demonstrates that the equilibration of D_2O in the various tissues of the endotoxin shocked dog is not attained in the 2 hour period of shock. It appears pertinent that the intestine and liver took up sufficient D_2O from the blood in the first 20 min., such that despite a subsequent gradual release paralleling the concentration, in blood neither reached equilibrium with the blood. The intestinal retention was most marked in this regard and is consistent with the previously reported sequestration of plasma tag in this issue. A

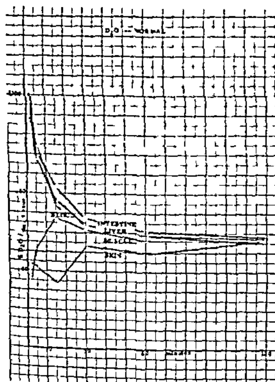


Fig. 1. The distribution kinetics of deuterium oxide in the blood and several tissues of the normal dog over a two hour period

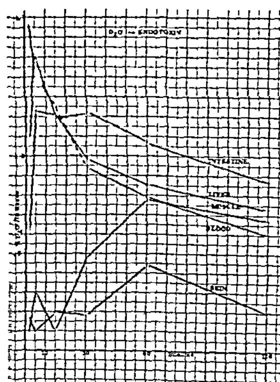


Fig. 2. The distribution kinetics of deuterium oxide in the blood and several tissues of the E. coli endotoxin shocked dog over a two hour period

comparison of the normal versus endotoxin blood disappearance curves shows the slower kinetic distribution of D_2O in the endotoxin-shocked animal

The bar graph in Figure 3 presents the average values for blood flow, calculated using D_2O distribution for both normal and endotoxin shocked dogs The most striking feature is the marked decrease of flow rates to each of the tissues studied following endotoxin administration The normal value for intestine of about 1 2 ml /min /gm is diminished to 0 4 ml /min while muscle, liver, and skin have an even greater proportionate lessening of flow

The changes encountered using antipyrine were qualitatively the same but quantitatively not as significant

DISCUSSION

The equilibration times for D_2O in antipyrine appeared prolonged in the endotoxin shocked animal Certain tissues, notably the intestine and liver appear to reach an initial equilibrium with the blood concentrations of D_2O and antipyrine These substances are rapidly taken up from the blood when the blood concentration is high, then lost back to the blood as other tissues with lower flows take up the tags from the blood The curves for liver and intestine followed the blood concentration curve down until final equilibrium was established throughout the body

Calculations based on D_2O disappearance from the blood stream in the first 2 min indicate that the tissue with the highest normal flow per gram of tissue, intestine, decreased proportionately less in endotoxin shock, than those tissues having normally lower flows Of the tissues examined, the skin is slowest to equilibrate and the muscle intermediate in keeping with their respective blood flows

The derived flows are approximations More samples in the first few minutes would add validity to the calculations

SUMMARY

The kinetic distribution of D_2O and antipyrine over a 2 hour period of time in blood and tissues of the normal and endotoxin shocked dog have

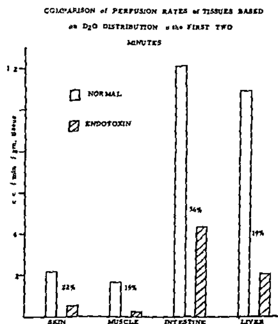


Fig 3 A comparison of the blood flow in ml /min to the various tissues in normal and endotoxin shocked dogs

been studied. In the normal studies liver and intestine were found to have high flow rates and pick up these tags very rapidly from the blood. However in endotoxin shock the perfusion rates of all tissues were markedly decreased and D_2O equilibration between blood and tissues failed to take place during the 2 hour observation period.

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THE ROLE OF THE SMALL INTESTINE IN HEMORRHAGIC SHOCK*

JOHN M. MCRAE, JR., WATTS R. WEBB, SAE S. LEE, JAMES D. HARDY AND JAMES C. GRIFFIN, JR.

Numerous investigators have called attention to the bloody diarrhea and the congestion and ulceration of the intestinal mucosa that occur during hemorrhagic shock[1] but have not stressed the potential deleterious effect of this fluid loss. Others have confirmed that irreversible hemorrhagic shock could be prevented by perfusing the liver of the shocked dogs with oxygenated blood.[2] Lillehei[3] demonstrated that perfusion of the superior mesenteric artery, either with or without an Eck fistula, thus perfusing primarily the small bowel and right colon, was more effective in preventing irreversible hemorrhagic shock than perfusion of the liver. These dogs of Lillehei did not show the mucosal congestion and necrosis which had been noted in the control animals. A "hemin" pigment was present in higher concentration in the plasma of the non-survivors than in the survivors, and this pigment increased coincident with the development of irreversibility.[4]

The large amounts of bloody fluid within the bowel and the bloody diarrhea suggested to us that the additional blood and fluid loss from the bowel contributed to the irreversibility of hypovolemic shock. The following experiment was devised to further delineate the role of the small bowel in hemorrhagic shock.

METHOD

Thirty-nine healthy, fasting, mongrel dogs weighing around 15 kilograms were sedated with sufficient sodium thiopental intravenously to keep them quiet during the initial procedure. Plastic catheters were inserted into a femoral artery and attached to an arterial reservoir with a side arm leading to a mercury manometer. In the experimental group the entire small bowel

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* From the Department of Surgery, University of Mississippi Medical Center, Jackson. Supported by the National Institutes of Health Grant Number RG-4745(C).

was resected from the ligament of Treitz to the ileocecal junction. The ileocecal junction was inverted and the proximal jejunum was exteriorized to allow the escape of gas. No fluid was given to replace that lost during this initial surgery. After oozing from the operative sites appeared controlled, both groups were heparinized with 10 milligrams of heparin per kilogram of body weight. The dogs were then bled into the reservoir, which was adjusted to maintain a constant pressure of 30 millimeters of mercury. As the experimental preparations are not compatible with prolonged existence, two primary observations were made: first, the time of reversal of blood flow from the reservoir, and second, the total period of survival. Serial hematocrit and pH determinations were made as were recordings of the pulse and respiration. All experiments were performed in an air-conditioned room.

OBSERVATIONS

The time of reversal in the 15 control dogs averaged 1 hour and 36 minutes, while the 18 experimental animals with the small bowel resected averaged 2 hours and 18 minutes ($P < .005$). The survival time of the control series averaged 5 hours and 40 minutes, as compared to 7 hours and 15 minutes in the resected series ($P < .05$). The nonresected dogs all died within 15 minutes after completely taking back their blood. Of the 19 resected animals, however, 10 lived for 30 minutes or longer after complete uptake, 6 for an hour or longer, and 2 for 2 hours.

Bleeding volumes from the control animals averaged 48.6 cc./kg. body weight, as opposed to 37.4 cc./kg. of body weight in the experimental dogs. The blood loss in the abdominal cavity of the resected group at autopsy ranged from 50 to 200 cc. with an average of 10 cc./kg., which would approximately equalize the bleeding figures. In addition this blood loss in the peritoneal cavity in the resected dogs was not available for return to the vascular system as was that comparable volume in the arterial reservoir of the control.

Bowel weights were quite variable, but in the resected series averaged 368 grams. In the control series the average weight of the small intestine removed

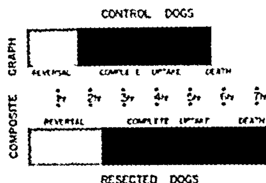


Fig 2. Composite graph of both groups showing average times of reversal uptake and death

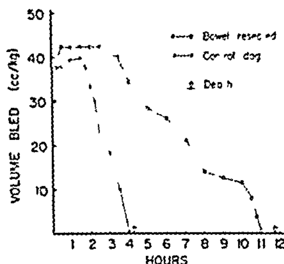


Fig 3 Graph of course of paired control (dog 1) and experimental animal (dog S 2)

at death was 170 grams In 6 additional control dogs in which the ileocecal junction had been clamped prior to the inception of hemorrhagic shock, the weight of the small intestine was found to average 596 grams with 200 cc of bloody fluid in the lumen of the bowel The hemoglobin content of the fluid ranged from 1 3 to 10 2 gm % with an average of 6 9 Bloody fluid was always present in the duodenum in both series and in the small and large bowel of the controls It was a less constant finding in the colon of the resected animal

The hematocrits in both groups were comparable initially except for 2 of the resected dogs which had hematocrits of 61 and 67 respectively As expected, these dogs did quite poorly In both series the hematocrit showed a fall to the time of reversal and a rise thence until death, except in the 2 dogs with high initial hematocrits in which the hematocrits constantly fell Otherwise no significant difference was found between the two series in these or in pH or pulse or respiratory changes

DISCUSSION

MacLean and associates have demonstrated a marked loss of fluid into the intestine following endotoxic shock with the small bowel weight rising as much as 196 to 770 grams in the hour following the endotoxin The liver weight likewise rose, as did the hematocrit These fluid losses easily accounted for the hypotension observed

The role of bacteria cannot be discounted in shock but it would seem that the small bowel resections done here did not affect the bacterial population significantly, as the more contaminated large bowel remained intact In addition though the stomach and the large bowel in both series of dogs demonstrated some mucosal changes, they were not as pronounced as the changes in the small bowel, which would suggest other factors were more significant than bacteria Nor would small bowel resection seem to prevent the production of a hemin pigment from the remaining bowel perfused by the superior mesenteric artery

SUMMARY

From our experiments it would seem that the small bowel is the locus minoris resistentiae, and during hemorrhagic shock a marked increase in intestinal capillary permeability occurs, with mucosal edema, ulceration and loss of vital intravascular fluid into the intestinal lumen This additional intralumenal loss of blood and plasma as the result of the peculiar susceptibility of the intestinal capillary bed to ischemia appears to be one of the significant lethal factors in hemorrhagic shock

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INTESTINAL FACTOR IN IRREVERSIBLE HEMORRHAGIC SHOCK *

LOUIS H POWERS AND PAUL R SCHLOERB

Hemorrhagic shock with its subsequent irreversible physiological effects has been known and studied for years, with ischemic injury to the liver and brain mainly implicated as the important factors in the irreversible shock state However, the predominant lesions of experimental animals succumbing to the effects of irreversible hemorrhagic shock are mucosal congestion and necrosis of the small and large bowel Recent work [1] has demonstrated that this irreversible mechanism in dogs could be largely prevented by perfusion of the superior mesenteric artery at near normal arterial pressure, while the general systemic arterial circuit was maintained at shock levels Previous work [2 3] has shown that bacterial endotoxins liberated from the intestine play an important role in the irreversibility of hemorrhagic shock, and that intestinal antibiotics have a protective effect if given prior to the induction of hemorrhagic shock

With this background, extirpation of the small and large bowel was carried out prior to the induction of irreversible hemorrhagic shock to evaluate its role in the dynamics of irreversible shock

METHOD

Thirty healthy, adult mongrel dogs were utilized with 15 of the animals serving as controls All dogs were fasted, except for water *ad lib*, for 12 to 16 hours prior to the procedure ' emedication was given before anesthetiz

* From the Department of Sur[illegible] ity of Kansas School of Medicine, Kansas City Kansas Supported by the Surger[illegible] t Fund

ing with intravenous pentobarbital, 25 mg./kg. All dogs were supported with endotracheal oxygen at 5 liters per minute for the operative part of the experiment.

In the enterectomized group of dogs, cannulation of the left femoral artery was done for continuous mean arterial blood pressure observations using a mercury manometer. Following this, median laparotomy was carried out, using aseptic technique, with ligation of the superior mesenteric artery at its origin from the aorta. The intestine was then extirpated from the proximal duodenum to just above the anus The proximal end of the duodenum was brought out as a duodenostomy at the time of closure. The right femoral artery was then cannulated for bleeding the dog to desired shock levels and for reinfusion of the blood. Withdrawn blood was kept in a sterile flask in a water bath at 37°C. Four milligrams of heparin were added per 100 ml. of blood

The same procedure was carried out on the controls, except for the ligation of the superior mesenteric artery and extirpation of the bowel. An attempt was made to dissect and handle the intestine in a comparable manner, except for its removal. A constant operating time of 1½ hours was maintained in both the experimental and control animals

All dogs were subjected to the effect of standardized irreversible hemorrhagic shock after the operative part of the experiment. A modification of the technique of Wiggers [4] was used. Both the enterectomized and control groups were kept at a mean arterial blood pressure of 50 mm. Hg for 1½ hours and 35 mm Hg for ½ hour. After reinfusion of the warmed and heparinized blood, the dogs were observed and survival time was recorded. Blood pressures were recorded at 15 minute intervals until either stabilization or death of the dog. Hemoglobin and hematocrit determinations were obtained before the experiment and after reinfusion of the blood. A record was maintained of the bleeding volumes, and autopsies were performed on all dogs.

RESULTS

The experimental data, subjected to the "t" test are summarized in Table 1.

Table 1 Intestinal Factor in Irreversible Hemorrhagic Shock

NUMBER OF DOGS	CONTROL 15	ENTERECTOMY 15	P
Bleeding Vol—ml /kg	36 ± 6	33 ± 6	0 30
B P Change after Reinfusion—mm Hg	—30 ± 13	—13 ± 8	0 001
Survival—Hours	8 9 ± 7 6	19 1 ± 12.2	0 013
Hematocrit Change	—4 1 ± 3 5	—8 4 ± 3 1	0 01

The enterectomized group of dogs survived a mean of 19 1 ± 12 2 hours after reinfusion of the withdrawn blood, while the control group lived a mean of 8 9 ± 7 6 hours The mean arterial blood pressure returned more nearly to the prebleeding level in the experimental group than in the control

For the most part, the dogs followed the usual pattern of demise from the irreversible effects of hemorrhagic shock. There was elevation of the blood pressure following reinfusion of the blood followed by a gradual decline until death. At autopsy the intestines of the control dogs showed the usual mucosal congestion, edema and necrosis typical of dogs dying of irreversible hemorrhagic shock. The spleens of dogs in both groups were small and contracted

DISCUSSION

MacLean *et al.*[5] (1956) have demonstrated, using gram negative endotoxins, that the gut and liver weights increase during the course of irreversible endotoxin shock. Characteristically, the liver weight rises abruptly, associated with an increased portal pressure, and then slowly returns to normal. The intestinal weight rises gradually over a 4 to 6 hour period until death ensues Aust *et al.*[6] reported the finding of plasma sequestration in the intestine of endotoxin-shocked dogs to explain this increased intestinal weight. They favored the concept that capillary permeability is increased to the point where leakages of large protein molecules into the interstitial spaces can occur.

The above findings are consistent with the edematous, congested intestine found at autopsy in dogs dying of irreversible hemorrhagic shock and with the experimental observations and results found in this study. It is believed that the increased survival time of the enterectomized dogs and their increased pressor response after reinfusion of the withdrawn blood can be attributed to the enterectomy itself. The bacteremic or endotoxin factor, as well as plasma sequestration or "pooling" into the intestine is largely averted by its gross removal.

The higher hematocrit values of the control series support the explanation of plasma sequestration. The difference may have been greater if serial hematocrit readings were taken up to the time of death of the dogs.

Whether there is direct invasion of the blood stream by bacteria from the bowel of the shocked dog is debatable. Fine[3] believes that resistance to bacteria and bacterial toxins declines during the shock phase. He states that antibiotics given prior to the induction of severe or prolonged hemorrhagic shock prevent the development of irreversibility to transfusion and increases the survival rate from 20% to 65% or better. Dogs transfused after exposure to 2 hours of hemorrhagic shock usually recover. An intravenous dose of bacteria, innocuous to the normal dog, is fatal in such dogs, whether given during the 2 hour period, or up to 24 hours thereafter. Normal resistance returns after 48 hours. However, of 192 blood cultures taken at intervals during shock up to the time of death, only 4 were positive; 2 for clostridia and 2 for Pseudomonas.

SUMMARY

1. Using controlled conditions and standardized irreversible hemorrhagic shock, intestinal extirpation in dogs resulted in greater survival time, greater return of blood pressure toward normal, and a lower hematocrit.

2. Removal of the bowel as a site of plasma sequestration as well as elimination of the bacteremic and entoxic factors may explain these observations.

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THE EFFECT OF ACUTE ADDITION ACIDOSIS ON EXPERIMENTAL HEMORRHAGIC HYPOTENSION *

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The reactivity of blood vessels is known to be affected by neurogenic stimuli catechol amines (epinephrine and nor-epinephrine) electrolytes steroid hormones of adrenal and gonadal origin and the acid base setting of the tissues Hypotension has been reported in postoperative patients who have experienced respiratory acidosis during operation [1, 2] This hypotension cannot be explained on the basis of blood loss or other operative factors One possible cause for this hypotension is that the response of the blood vessel to the stimulation of the catechol amines has been diminished by the acidotic condition of the patient [3, 4] In addition myocardial function becomes a critical factor in the maintenance of pressure flow during hypotension acid base factors may adversely affect cardiac output

Shock with inadequate tissue perfusion is regularly followed by the development of a metabolic acidosis because of the accumulation of anaerobic metabolic products in the tissues which are acid in composition The more severe and prolonged the shock episode is the more likely that the acidosis produced will be severe Because this acidosis might be the source of difficulty in blood pressure maintenance in the occasional critically ill patient requiring vasopressors the following experiments were undertaken They were designed to demonstrate the effect of an acute addition metabolic acidosis on cardiac function and peripheral vascular response (as indicated by peripheral resistance) in the experimental animal subjected to hemorrhagic hypotension

METHOD

Unanesthetized splenectomized dogs weighing 10 to 22 3 kg were used throughout these experiments Using 2% local procaine anesthesia the femoral artery and vein were cannulated and the dog placed in a Pavlov box The following baseline determinations were carried out arterial blood was drawn under oil for determination of the pH using the Cambridge research model pH meter at a constant temperature of 38 0°C The total plasma CO_2 (tCO_2) was determined by the method of Van Slyke and Neill [5] The blood

* From the Department of Surgery Harvard Medical School and the Peter Bent Brigham Hospital Supported in part by the Subcommittee on Metabolism in Trauma Advisory Committee on Metabolism Office of the Surgeon General Department of the Army

bicarbonate and pCO_2 were calculated using the Henderson Hasselbach formula with pK' taken as 6 10 Serum sodium and potassium were determined using a Baird flame photometer and the serum chlorides were determined using the method of Wilson and Ball [6]

The mean blood pressure was read from a mercury manometer connected to the femoral arterial cannula Cardiac output was determined using the Cr^{51} tagged red cell dilution method [7] The red blood cells were tagged with Cr^{51} by the method described by Read [8] Total peripheral resistance was calculated as resistance units using the formula

$$\text{Resistance Units} \simeq \frac{\text{B P times 60 sec}}{\text{Cardiac Output in cc /min}}$$

Electrocardiograms were recorded from leads I, II, III, and AVL preceding each cardiac output The blood pressure, cardiac output, and calculated resistance constituted the hemodynamic observations made in these animals

The dogs were divided into three experimental groups according to the following protocols

Group 1. Acidosis-Hemorrhage-Bicarbonate Replacement. This group consisted of 5 animals which received 8 mEq /kg body weight of 0 7 N HCl as an intravenous infusion over a two and one half hour period of time At the completion of the infusion, acid base data, serum electrolytes, electrocardiograms and hemodynamic studies were repeated The animals were then subjected to a graduated arterial hemorrhage consisting of 2% of the animal s body weight occurring over a 30 minute time interval The blood pressure was observed during the following 60 minutes of hypotension and the above measurements repeated Concentrated $NaHCO_3$ was then administered in a dosage of 8 mEq /kg body weight over a 15 minute period of time, and following an additional 15 minute period of equilibration the observations listed above were made again

Group 2. Acidosis-Hemorrhage Saline Replacement. This group consisted of 4 dogs which were made acidotic and hemorrhaged exactly as in Group 1 Following hemorrhage, the acidosis was not corrected in this group These animals received concentrated NaCl 8 mEq /kg body weight in a volume equal to what it would have been had it been concentrated $NaHCO_3$ The concentrated NaCl was administered at the same time interval as was the $NaHCO_3$ in Group 1 This occurred 90 minutes after the start of the graded hemorrhage Blood sampling, electrocardiograms, and hemodynamic observations were made exactly as in Group 1

Group 3. No Acidosis-Hemorrhage-5% Dextrose in Water Replacement This group consisted of 4 dogs which served as controls Instead of receiving hydrochloric acid to produce an addition acidosis as in Groups 1 and 2, these animals were infused with concentrated NaCl 8 mEq /kg body weight in a volume equal to what it would have been had it been 0 7 N HCl The NaCl was infused over a 2½ hour interval Hemorrhage was carried out exactly as in Groups 1 and 2 Following this hemorrhage the Group 3 animals received a volume replacement of 5% dextrose in water This volume was equal to what it would have been had it been concentrated $NaHCO_3$ or NaCl, and was also administered 90 minutes following hemorrhage Blood sampling electrocardiograms, and hemodynamic observations were made exactly as in the preceding groups

RESULTS

Group 1. Acidosis-Hemorrhage-Bicarbonate Replacement. The laboratory results from the Group I animals are tabulated and appear in Table I. The blood pH values varied between 6.92 and 7.12 following the infusion of 0.7 N. HCL The tCO_2 dropped to between 4 5 and 9 3 mM/L. The pCO_2 was reduced in all cases. The serum K and Cl rose in all cases and there was a variable response in the serum Na level with acid infusion. The cardiac output dropped below the control value, an average of 27 2% for the group, while the peripheral resistance rose in all cases. Following the graded hemorrhage there was a further drop in cardiac output below the control value averaging 58 4%. There was a corresponding rise in the total peripheral resistance. Following the correction of the acidosis by equimolar administration of concentrated $NaHCO_3$, the pH returned to within normal limits in all dogs. The serum K dropped to normal in all cases. There was a drop in the serum Cl in all animals. There was a variable blood pressure response. In all animals there was a rise in cardiac output over the value obtained following acidosis and hemorrhage averaging 35.8% for the group. There was a fall in total peripheral resistance in all dogs following $NaHCO_3$ administraiton.

Group 2. Acidosis-Hemorrhage-Saline Replacement. The data from Group 2 are presented in Table 2. One dog expired following acid infusion. As in

Table 1. Acidosis Hemorrhage-HCO_3-Replacement

LEGEND

pH	B P.	Na^+
tCO_2	C O	K^+
pCO_2	R	Cl

	BASELINE			PRE HEMORRHAGE			POST HEMORRHAGE			HCO_3^- REPLACEMENT		
	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES
DOG 1	7 37	120	126	6 98	128	145	7 06	85	150	7.39	98	156
14 1 kg ♂	24 9	2758	3 7	6.5	2165	4 6	10.2	1026	4 6	22 8	1399	3 1
	42.2	2 6	112	25.2	3.5	130	34.2	5 0	149	36 9	4.2	139
DOG 4	7.31	155	135	6 92	150	138	7 01	130	136	7.35	130	152
13.2 kg ♂	24 9	2322	4 0	4 7	1731	5 0	5.3	955	5 7	12 6	4297	3 6
	48 0	3 9	108	20.5	5.2	128	19 3	8.2	124	22.2	1 8	116
DOG 7	7.35	105	142	7 09	105	141	—	92	140	7 49	76	152
11 8 kg ♂	18.8	3245	4 4	7 8	2605	4 9	7 6	1461	5 6	16.5	2408	3 7
	33.3	1 9	104	24 1	2 4	127	—	3 8	120	21.5	1 9	102
DOG 10	7 33	135	148	7 12	140	144	7 11	110	145	7 40	105	155
11 4 kg ♂	19 0	3464	3.3	9 3	2244	4 4	8 8	1416	4.2	19 7	1595	3.3
	35 1	2 3	113	26 9	3 7	127	26 0	4 4	128	31.2	3 4	123
DOG 13	7 38	134	151	6 94	135	142	6 98	95	139	7 42	108	155
20 9 kg ♀	21 3	2940	4 0	4.5	1931	5.5	5 3	1292	5.3	19 6	1699	3 1
	35.2	2 7	115	18 9	4.2	134	20.5	4 4	132	29 8	3 8	128

Laboratory data from Group 1 dogs undergoing addition metabolic acidosis, hemorrhage and sodium bicarbonate replacement

Table 2 Acidosis Hemorrhage Saline Replacement

LEGEND	BASELINE			PRE HEMORRHAGE			POST HEMORRHAGE			SALINE REPLACEMENT		
pH · B P · Na^+ / $_tCO_2$ · C O · K^+ / pCO_2 · R · Cl	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES
DOG 2	7 37	90	155	6 89	100	148	6 96	72	141	6 96	92	131
18 2 kg ♀	19 3	3425	3 1	4 8	2152	—	5.2	975	5 0	—	1735	4 4
	32 7	1 6	115	22 3	2 8	139	21 0	4 4	139	—	3 2	152
DOG 5	7 34	115	152	6 93	110	142	6 88	30	150	6 98	95	158
16 0 kg ♀	20 6	2662	3.2	4 2	1534	5.5	2 8	680	9 2	3.57	1800	5 8
	37 2	2 6	105	17 2	4 3	129	13.2	2 7	132	13 8	3.2	121
DOG 8	7 39	130	142	7 05	140	143	7 08	120	142	7 05	115	151
16 4 kg ♂	23 8	—	3 8	6.5	3255	4 7	7 1	1230	5 2	7.21	4077	4 0
	38 6	—	110	21 8	2 4	127	22 4	5 9	124	24.2	1 7	138
DOG 11	7 33	145	140									
13 6 kg ♂	20 5	4039	4 2	(DOG EXPIRED)								
	37 8	2.2	112									
DOG 14	7 33	110	143	7 05	120	143	7.04	56	142	7 09	100	155
19 5 kg ♀	20 9	2168	4 2	9 0	2105	5 2	5 3	678	5 2	9 0	1656	6 0
	38 6	3 0	112	30.2	3 4	130	18 1	5 0	130	27 8	3 6	149

Data from Group 2 dogs undergoing addition metabolic acidosis hemorrhage and saline replacement

the preceding animals there was a marked fall in pH and tCO_2 and a rise in the serum K and Cl associated with the administration of the HCl The cardiac output likewise fell and the peripheral resistance rose The average fall in cardiac output below the control value following acid infusion was 27 5% for this group Following hemorrhage, there was a further drop in the cardiac output below the control value of 71 6% for the group Following the graded hemorrhage, dog 5 became extremely acidotic and hyperkalemic with a pH of 6 88, a tCO_2 of 2 8 and a serum K of 9 2 mEq Following the administration of concentrated saline solution, there was little change in the animal s acid base status There was a fall in the serum K in 3 of 4 animals In all dogs following saline infusion there was a rise in the cardiac output over the value obtained following acidosis and hemorrhage of 58 7% for the group and a decline in total peripheral resistance in 3 of the 4 dogs

Group 3. No Acidosis-Hemorrhage-5% Dextrose in Water Administration Group 3 data are presented in Table 3 One animal convulsed and suddenly died during the graded arterial hemorrhage There was a slight decline in pH and tCO_2 associated with the infusion of concentrated NaCl There was an over all rise in cardiac output of 18% over the control value with the NaCl

infusion, and the total peripheral resistance dropped in 3 out of 4 animals. The serum Na and Cl remained the same or increased in each instance. The serum K was essentially unchanged. Following graded arterial hemorrhage, there was a drop in cardiac output below the control value of 37.8% for the group. The total peripheral resistance rose in each instance. The blood pressure did not appear to drop as much as that observed in the acidotic animals subjected to hemorrhage. The final administration of 5% dextrose in water to control the volume that would have been administered as $NaHCO_3$ resulted in no change in acid base or electrolyte composition. There was a further over all drop in cardiac output below the value obtained following saline infusion and hemorrhage of 5.2% since the drop in output was greater in the 2 animals showing a decline than in the remaining 2 animals showing a slight rise in output. The resistance values varied inversely with the cardiac output following the administration of dextrose in water.

DISCUSSION

It would appear from this study that the blood vessel wall in the unanesthetized dog is able to respond by contraction and maintenance of the total peripheral resistance even though the extracellular fluid is severely acidotic. The principle difference between acidotic and nonacidotic dogs subjected to a hemorrhagic challenge lies in the cardiac response. Acidosis alone

Table 3. No Acidosis-Hemorrhage 5%-D/W Replacement

LEGEND:

pH	B P	Na^+
tCO_2	C O	K^+
pCO_2	R	Cl

	BASELINE			PRE HEMORRHAGE			POST HEMORRHAGE			5% D/W (CONTROL)		
	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES	ACID BASE	HEMODYNAMICS	ELECTROLYTES
DOG 3	7.36	145	136	7.32	140	141	7.32	100	132	7.32	100	135
22.3 kg ♀	23.1	2749	3.5	21.3	4226	3.5	17.0	2138	3.0	17.3	1778	3.2
	40.0	3.2	102	40.2	2.0	111	32.1	2.8	111	32.6	3.4	116
DOG 6	7.39	150	146	7.33	160	154	7.33	110	156	7.32	94	153
15.5 kg ♂	21.9	3034	3.9	20.0	3757	3.3	17.9	1339	3.5	17.9	1605	3.4
	35.5	2.9	114	37.0	2.6	114	33.1	4.9	127	33.8	3.5	125
DOG 9	7.42	130	142	7.38	132	158	(DOG EXPIRED)					
20.5 kg ♀	16.4	2430	3.8	14.1	1333	3.8						
	24.9	3.2	110	23.4	5.9	129						
DOG 12	7.37	122	143	7.30	125	151	7.30	86	153	7.31	80	150
10.0 kg ♂	25.1	1813	3.2	22.3	3387	3.7	18.6	1183	3.3	18.0	1217	3.0
	43.2	4.0	113	44.0	2.2	126	36.7	4.4	127	34.7	3.8	120
DOG 15	7.38	105	148	7.36	140	155	7.37	134	155	7.33	125	149
17.3 kg ♂	22.4	3930	3.9	19.8	2559	3.8	19.1	2430	3.9	18.6	2116	3.9
	37.1	2.3	114	34.3	3.3	124	32.3	3.3	124	34.4	3.8	120

Laboratory results from Group 3 control dogs

produced a moderate decline in the cardiac output and when hemorrhage is superimposed on these already sick animals, the drop in output is severe. In only 1 animal, dog 5, in which acidosis was severe with pH 6.88 and tCO_2 2.8 was there evidence of impending circulatory collapse. This was the only acidotic dog in the series which showed a drop in resistance during the period of *hemorrhagic hypotension.*

The rise in serum K associated with acute addition acidosis was enough to produce electrocardiographic changes in some of these animals. In 1 dog following acidosis and hemorrhage the serum K was 9.2 mEq. It may be that some of the myocardial effects of acidosis are due to hyperkalemia. When the stress of hemorrhage is added to the already sick myocardium, severe reduction in cardiac output occurs with a corresponding drop in blood pressure. The administration of $NaHCO_3$ to correct the acidosis resulted in the correction of hyperkalemia in all animals. It is interesting to note, however, that the administration of hypertonic NaCl, which also reduced serum K levels but did not correct the acidosis, [illegible]
tion as indicated by impro [illegible]
infusion the rise in cardiac [illegible]
and hemorrhage was 35.8% as against a rise of 58.7% in those animals receiving hypertonic NaCl. In the Group 3 animals receiving dextrose in water infusions to control the volume administration of $NaHCO_3$ or NaCl, there was a decline in the cardiac output below the value obtained following saline administration and hemorrhage of 5.2% for the group *despite the volume* replacement in an animal with an acute volume reduction.

These data from the dog suggest that in the critically ill patient in shock, therapy should include not only volume replacement but also the correction of any existing acidosis by the use of alkalinizing solutions whenever this is practical. The myocardium will thus be placed in an optimum acid-base setting to function efficiently and any tendency toward the hyperkalemia of severe metabolic acidosis will be reduced or eliminated.

SUMMARY

1. The acid-base, electrolyte, electrocardiographic and hemodynamic response of dogs subjected to an acute addition metabolic acidosis and hemorrhagic hypotension has been studied.

2. The acidotic dog develops a hyperkalemia as well as a reduction in cardiac output. The electrocardiographic changes resemble those produced by hyperkalemia.

3. The acidotic animal when subjected to a hemorrhagic challenge develops a marked drop in cardiac output and blood pressure but compensates well for this lowered flow by peripheral vasoconstriction and elevation of the total peripheral resistance and thus the blood pressure.

4. The hyperkalemia associated with addition acidosis and hemorrhagic hypotension can be quickly corrected by the administration of $NaHCO_3$.

5. The importance of maintaining normal acid-base balance in the management of clinical patients with shock is pointed out.

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METABOLIC ASPECTS OF HEMORRHAGIC SHOCK *

I Changes in Intermediary Metabolism During Hemorrhage and Repletion of Blood

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The initiating disturbance in hemorrhagic shock is a reduction in circulating blood volume produced by a loss of blood The physiologic disturbances produced by blood loss have been studied extensively over many years [1] Today almost all investigators agree that the consequences of hemorrhage can be prevented or successfully overcome by the prompt use of transfusion therapy When death does occur, it seems to result from disorganization at the cellular and enzymatic level A clear definition of the nature of the metabolic disorganization that occurs in hemorrhagic shock might suggest means of reviving or sustaining the energy yielding reactions essential to life until transfusion therapy permits reestablishment of normal metabolic pathways The present preliminary study was designed to measure several parameters of intermediary metabolism before and during hemorrhagic hypotension and following reconstitution of the blood volume in dogs that eventually succumbed to shock

METHOD

Hemorrhagic shock was produced in 9 mongrel dogs by the Selkurt modification of Wiggers technique [2] The animals were fasted overnight Anesthesia was produced by the intravenous administration of 30 mg/kg body weight of sodium nembutal

A small polyethylene cannula was inserted into each femoral artery One cannula was connected to a mercury manometer for measurement of mean arterial pressure The other cannula was led to a large siliconized glass reser

* From the Department of Surgery Western Reserve University School of Medicine and University Hospitals of Cleveland Cleveland Supported in part by grants from the U.S P H.S grant #A 1253 and from the Meade Johnson Company

voir which was used to store the removed heparinized blood. A larger polyethylene cannula was inserted into each femoral vein and passed into the inferior vena cava. One cannula was used for collection of blood samples; the other was used for administration of the radioactive iodinated human albumin for blood volume studies. Sodium heparin (2 mg./kg. body weight) was given intravenously after insertion of the cannulas.

After withdrawing a control sample, the clamp was removed from the arterial cannula which led to the reservoir. It was found that an average hemorrhage of 37 ml. of blood per kg. of body weight was required to reduce the mean arterial pressure to 50 mm.Hg. Zero time was established when the pressure stabilized at 50 mm.Hg. Samples of venous blood were then drawn for chemical analysis every thirty minutes until the animal was transfused. After the blood pressure was maintained at 50 mm.Hg for ninety minutes, an average additional 10 ml. of blood per kg. body weight was withdrawn to reduce the pressure to 30 mm.Hg. When there was a spontaneous uptake of blood by the animal from the reservoir (average 5 cc./kg.), it was felt the physiological compensation had failed and the remaining blood was returned forcibly. This was followed by a prompt elevation in arterial pressure. A venous sample was collected at this point and subsequent samples were collected at thirty minute intervals. On two occasions, the volume of blood returned to the dog equalled the volume bled into the reservoir plus the cumulative volume withdrawn for samples (average 5 ml./kg.). The results in these cases did not differ significantly from the ones in which the cumulative volume of sample blood was not replaced. The blood samples were analyzed for glucose, pyruvic and lactic acids and serum inorganic phosphorus by methods previously described.[3] Blood volume was determined before hemorrhage, during the period when the arterial pressure was at 30 mm.Hg, and approximately two hours following the return of the reservoir blood, by the method of Storaasli *et al.*[4] Venous pH was determined periodically by the method of Craig *et al.*[5]

Three control experiments were performed: 2 dogs received nembutal anesthesia but were not bled. One dog was bled as a control for the severity of shock at 90 mm.Hg for 90 minutes and at 50 mm.Hg for 60 minutes. This dog survived following transfusion.

RESULTS

A reduction of the mean arterial pressure to 50 mm.Hg caused an average rise in the levels of blood glucose, pyruvic and lactic acids and serum inorganic phosphorus of 216, 1.88, 85.3, and 3.14 mg. % respectively. The lactic/pyruvic (L/P) ratio rose to 34 from a control of 13. The average venous pH fell from 7.31 to 7.09.

A further reduction of mean arterial pressure to 30 mm.Hg caused an additional rise in blood glucose, pyruvic and lactic acids of 71, 0.91 and 39.8 mg. % respectively, but there was no significant change in phosphorus The L/P ratio rose to 37 and the venous pH fell to 6.97. The average time that the animals remained at this pressure was one hour and six minutes.

The return of blood caused a prompt elevation in mean arterial pressure to 105 mm.Hg. This was followed by a decline in pressure in all animals until death. The average duration of survival after transfusion was 5 hours and 13 minutes with a range from 1 hour and 52 minutes to 12 hours.

The return of the withdrawn blood caused a prompt reduction in the level of blood glucose but no immediate significant change in the other values. There was a gradual return of all parameters toward the control or preshock values despite the posttransfusion decline in blood pressure. The blood glucose reached the fasting level approximately 2 hours following transfusion. The phosphorus values remained significantly elevated for one hour after transfusion.

Although there was a decline in the pyruvic and lactic acids following transfusion, the values remained elevated above the control level until the animal died. Only one dog had a normal pyruvic and lactic acid level at the time of death. This dog survived the longest of the entire group. The average L/P ratio fell to control levels after 3 hours due to a late rise in pyruvic acid rather than a further reduction in lactic acid.

The dog in which blood pressure was reduced to only 90 mm. Hg and then to 50 mm. Hg recovered after transfusion. This animal also had a rise in glucose, pyruvic and lactic acids and phosphorus and a fall in pH. In contrast to the changes found after more severe hemorrhage, all values returned to the fasting or control level after transfusion.

Nembutal anesthesia (30 mg./kg. of body weight) in two dogs did not produce a significant change in glucose or pH. There was a very slight rise in pyruvic and lactic acids and in one dog a slight late rise in inorganic phosphorus. The metabolic changes found during hemorrhagic shock have all proved to be significantly different from the fasting values and the nembutal anesthesia controls by application of the Fisher "t" test.

Figure 1 illustrates the mean values for 9 animals, all of which succumbed. The graph is plotted for 3 hours after transfusion. Although the mean pyruvic and lactic acid values remained elevated above control levels, the degree of elevation was not statistically significant after 3½ hours, possibly due to the small number of animals that survived beyond this period. Figure 2 illustrates the minimal metabolic changes that occurred with nembutal anesthesia.

Table 1 is a comparison of the metabolic changes during two periods of hypotension. The first hypotensive period occurred during hemorrhage when

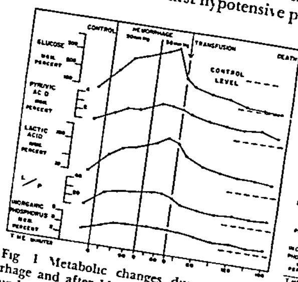


Fig. 1. Metabolic changes during hemorrhage and after blood replacement in dogs under nembutal anesthesia. (average of nine dogs)

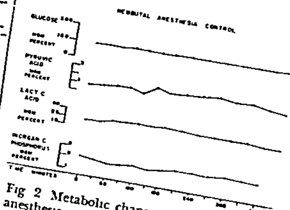


Fig. 2. Metabolic changes during nembutal anesthesia without hemorrhage. (average of two dogs)

Table I Comparison of the Metabolic Changes during Hemorrhagic Hypotension and Normovolemic Terminal Hypotension, (average of 9 dogs)

	CONTROL	HEMORRHAGE 50 MM HG	HEMORRHAGE 30 MM HG	EARLY	POST TRANSFUSION LOWEST LEVEL	POST TRANSFUSION TERMINAL (50–30 MM HG)
GLUCOSE MG %	92 8	309 1	380 3	220.5	80 5	87.2
PYRUVIC ACID MG %	1 10	2 98	3 82	3 70	1 85	2 22
LACTIC ACID MG %	14 3	99 5	139 0	129 8	45 1	49 8
INORGANIC PHOSPHORUS MG %	3 8	6 5	6 5	7 1	4 6	5 4
L/P	12 9	34 2	37 1	33 5	23 3	29 1
pH	7.28	6 96	6 87	6 99		7 09
BLOOD VOLUME ML per KG	82 3		50 6	88 8		

the mean blood volume was 51 ml /kg The second hypotensive period occurred following transfusion when the pressure fell as a terminal event The mean blood volume determined approximately 2 hours after transfusion was 89 ml /kg compared with the mean control volume of 82 ml /kg The metabolic changes were significantly less during the normovolemic terminal hypotension The average maximal rise from the lowest level reached after transfusion was for glucose, pyruvic and lactic acids and inorganic phosphorus 7, 0 37, 4 72, and 0 76 mg % respectively The pH did not change

DISCUSSION

Several excellent reviews have been written on the metabolic pattern that occurs with hemorrhagic shock [6, 7] The factor that initiates the metabolic changes during the period of hypovolemia is probably tissue anoxia [6] The increase in fasting blood sugar has been ascribed to glycogenolysis and gluconeogenesis from protein, lactic and pyruvic acids [7] An increased peripheral production and a decreased hepatic utilization have been held responsible for the rise in pyruvic and lactic acids [8] The shift to anaerobic metabolism with a rise in L/P ratio may lead to faulty energy production with a breakdown of high energy organic phosphorus compounds to inorganic phosphorus [7]

When the blood volume was restored by transfusion the arterial pressure

gluconeogenesis, depletion of hepatic glycogen, or a continued increase in peri

pheral glucose utilization. It is not possible to deduce from the present experiments which of these mechanisms was responsible. It was calculated that the dilutional effect from the lower level of glucose in the transfused blood (average 87 mg./100 ml.) was insufficient to account for the observed fall in glucose. The glucose level frequently started to fall before transfusion and continued to fall long after transfusion. Also transfusion did not cause a dilutional change in the levels of pyruvic and lactic acids, despite lower levels of these substances in the transfused blood.

There is evidence that hepatic removal of lactic and pyruvic acids is not impaired late in shock after transfusion.[9] Other studies indicate that lactic acid does accumulate late in hemorrhagic shock [8] but that the liver does not play a dominant role in this alteration.[10] Our results in animals that died after hemorrhage and transfusion indicate that pyruvate and lactate metabolism remains altered following transfusion but to a lesser degree than during hemorrhage. One animal in which the hemorrhage and reduction in pressure was less severe, had anaerobic metabolism during the hypotensive period but metabolism became aerobic following transfusion and the animal survived. One may speculate that the extraordinarily high lactic acid and low venous pH that occurs during hemorrhage may combine to upset vital enzymatic processes. The observation that administration of sodium bicarbonate may be of some therapeutic benefit is consistent with this speculation.[6] Such a disturbance in the presence of a continued alteration in pyruvate and lactate metabolism after transfusion may contribute to eventual cellular disorganization and death. It is unlikely that the persistent alteration in pyruvate and lactate metabolism is of itself a significant factor in the animal's death, because of the improvement that occurs after transfusion. The pyruvic and lactic acid levels returned to normal, however, in the control dog that survived.

The smaller changes in the metabolic pattern during the terminal period of hypotension may be due to restoration of a normal blood volume or it may indicate exhaustion or inhibition of the processes that produced the initial changes. This problem deserves further study and cannot be resolved by the presently available data.

The fall in pH has been ascribed to the accumulation of lactic and pyruvic acids plus other organic acids.[6] There is a return of pH toward the control level as the levels of pyruvic and lactic acids decline following transfusion. The extremely low levels of pH may be a reflection of the use of venous blood in the determinations.

The control studies with nembutal indicate that anesthesia did not play a significant role in the observed metabolic changes.

It had been concluded by many observers (and the present data support the concept) that no metabolic alteration has been found which, *per se*, can be held accountable for the failure to respond to transfusion after prolonged hypovolemia The present study was planned as a baseline. The several problems that have been raised require additional study.

CONCLUSIONS

1. Hemorrhagic hypotension followed by transfusion of the withdrawn blood caused a marked elevation of blood glucose, pyruvic and lactic acids, and serum inorganic phosphorus and a fall in venous blood pH.
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When the blood volume was restored by transfusion the arterial pressure rose to a normal range and the metabolic changes reverted toward the control values The fall in blood sugar may be due to cessation of glycogenesis and gluconeogenesis depletion of hepatic glycogen or a continued increase in peri

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2. Transfusion caused a prompt elevation in arterial pressure followed by a

gradual decline until death occurred in all animals The metabolic changes all reverted toward the control values following repletion of the blood volume

3 Pyruvic and lactic acid levels remained above the control levels until the animal died

4 The metabolic aberrations were significantly less pronounced during the terminal period of hypotension than during the initial period of hemorrhagic hypotension

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THE EFFECT OF METHYLENE BLUE AND ASCORBIC ACID IN HEMORRHAGIC SHOCK *

JOSEPH G STRAWITZ ROBERT L TEMPLE AND HELEN HIFT

Several studies within the past decade have suggested that an enzymatic defect in energy metabolism occurs in hemorrhagic shock [1] This contention is supported by recent work from this laboratory which has shown that injury develops at the mitochondrial level the site of cellular oxidation and energy production [2]

Since a number of substances are known to be active in *in vitro* oxidation reduction systems it seemed appropriate to study their effects in hemorrhagic shock on the premise that they might bridge areas of enzymatic injury

The rat was chosen as *the experimental animal since it lends itself to statistical evaluation* A standard shocking method was devised Methylene blue and ascorbic acid were tested with encouraging results

METHOD

Male albino rats of the Sprague Dawley strain weighing between 275 and 325 gm were maintained on an *ad libitum* diet of Purina rat chow They

* From the Dept of Surgery and Cardiovascular Research Laboratories University of Wisconsin Medical School Madison Supported by the Wisconsin Heart Association by the Department of the Army Office of the Surgeon General and by the National Heart Institutes of Health U S Public Health Service

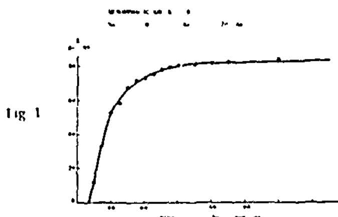


Fig 1

were fasted 12 hours prior to experimentation Each animal was immobilized with light ether anesthesia The distal 1 cm of the tail was amputated and about 15% of the theoretical blood volume i e 3 volumes % body weight, was removed over a period of 30 minutes The theoretical blood volume was taken to be 6 7 volumes % body weight [3]

Ninety minutes after the termination of tail bleeding the animal was once more lightly anesthetized A heparinized No 22 needle on a 5 ml syringe was inserted into the thoracic cavity adjacent to the xiphoid process The heart was located by careful probing and the needle thrust into it Additional blood was withdrawn to produce a final bleeding volume comprising about 60% of the total theoretical circulating volume i e 4 1 volumes % body weight

With the needle in place in the heart syringes were rapidly transferred and methylene blue 0 1 ml = 0 2 mg /100 gm body weight was injected in one series of rats ascorbic acid 0 1 ml = 1 mg /100 gm body weight in the other † Paired control animals were given corresponding volumes of 0 85% saline solution The volume thus injected in each case was compensated for by withdrawing an equivalent amount of blood at the time of heart puncture The behaviour of the animals the survival times and survival rates were then observed

Four lots of methylene blue Merck (obtained from Wm H Rorer, Inc in the form of 10 ml amounts of a 1% solution in sterile ampules for medical purposes) were used Fresh dilutions representing 0 2 gm % in physiological saline were prepared each time Two lots of ascorbic acid (not further identified) were obtained from the Krishell Laboratories Neutral 1% solutions in saline were prepared fresh each time

RESULTS

A series of 205 rats (Fig 1) subjected to the shock method described yielded an over all mortality rate of 82% Sixty to sixty five per cent of the deaths occurred within 20 to 70 minutes following the second bleeding This mortality rate approaches that observed in dogs subjected to Wiggers procedure [4]

Table 1 lists observations prior to death and at autopsy in both the rat and

† Levels of methylene blue ranging from 0 1 to 1 0 mg /100 gm body weight and of ascorbic acid ranging from 1 to 10 mg /100 gm body weight injected into unbled rats produced no signs of toxicity The doses chosen approximated therapeutic levels reported in the literature

dog The similarities are striking Intestinal hemorrhage is one of the more outstanding and constant findings

Table 1 A Comparison of Findings Associated with Irreversible Hemorrhagic Shock in the Dog and the Rat

	DOG	RAT
OBSERVATIONS PRIOR TO DEATH		
Hypotension	maintained	not measured
Sensorium	depressed	depressed
Respiratory Rate	increased	increased
Pallor	mucous membranes	mucous membranes retina extremities
POST MORTEM		
Intestinal Hemorrhage	present	present
Subserosal Hemorrhages	intestinal pleural pericardial	intestinal pleural
Congestion	liver kidney	liver kidney
Myocardial Flabbiness	present	present
Pulmonary Atelectasis	sometimes present	sometimes present

A suspension of normal rat liver mitochondria viewed under phase contrast is shown in Figure 2 Translucent spheres and rods are seen Figure 3 shows a suspension of such particles from "shock" rat liver There is a loss of rods, the mitochondria swell, become irregular and opaque Figure 4 shows similar changes observable in dog liver mitochondria as a result of hemorrhagic shock



Fig 2



Fig 3



Fig 4

Figure 5 is a plot of survival times and survival rates in a series of 29 control
valuation
t test P<
(X² test
P<0 008) In general the shocking procedure was tolerated better by the treated animals They regained conciousness and assumed their normal stance sooner after the final bleeding

The study with ascorbic acid was conducted in a similar manner using 31 controls and 38 experimental animals (Fig 6) The treated animals responded more favorably to shock The average survival time in the treated group was significantly lengthened (t test P<0 02) and the mortality rate significantly decreased (X² test P<0 019)

DISCUSSION

Several attempts at producing a standard type hemorrhagic shock in rats have been published [1-5] Some of the more promising methods have not worked well in our hands The major difficulty was the inability to withdraw sufficient amounts of blood from the tail as peripheral constriction progressed This has been overcome in the method described in this paper by supplementing the tail bleeding with a heart puncture A reproducible mortality rate and a consistent shock picture showing striking similarities to hemorrhagic shock in the dog has thus been obtained

Ascorbic acid was used by DePasqualini [6] in the treatment of hemorrhagic shock in guinea pigs and caused a decrease in mortality rate The effect was attributed to maintenance of capillary integrity rather than to redox potential

To the best of our knowledge the efficacy of methylene blue has not yet been evaluated in hemorrhagic shock It is described as having direct cardiovascular actions such as producing an increased cardiac output and vasoconstriction resulting in an elevation of blood pressure On the other hand methemoglobinemia is being treated with the dye on the assumption that it behaves as an oxidation reduction agent

It is probably premature to speculate whether the data reported in this paper are referable to the redox potentials of the drugs or to some of their other properties Certainly their action as plasma expanders can be ruled out because of their rather small molecular size

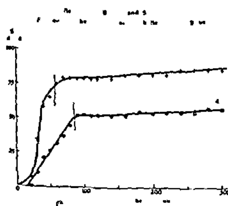


Fig 5

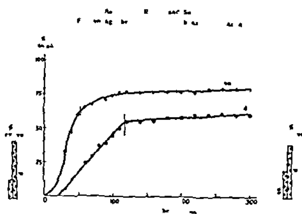


Fig 6

SUMMARY

A standard hemorrhagic shock method was produced in rats of the Sprague Dawley strain Animals subjected to this procedure were treated with methylene blue (0 2 mg /100 gm body weight) or ascorbic acid (1 mg /100 gm body weight) Paired control animals were given corresponding volumes of saline Both agents reduced the mortality rates significantly In addition ascorbic acid lengthened the survival times

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STUDIES IN HYPOTHERMIA AND ITS USE IN EARLY BURN THERAPY *

H HARLAN STONE AND J D MARTIN JR

During the past decade hypothermia has become increasingly popular as an adjunct to surgery especially that of the brain heart great vessels and liver *However its clinical application has far exceeded our understanding* of its basic physiology This study was designed to investigate the effects of hypothermia on electrolyte metabolism the blood vascular compartments the peripheral hematologic picture and certain routine clinical tests An evaluation of the advantageous use of hypothermia in burn therapy was then made using these measurements

METHOD

Forty three healthy adult dogs weighing 10 to 20 kg were divided into 3 groups

(1) Hypothermia controls—17 animals were subjected to hypothermia for 4 to 36 hours and then sacrificed at intervals of 12 to 14 days
(2) Burn controls—8 dogs received 30% second degree burns and were sacrificed at intervals of 2 to 28 days
(3) Burn experimentals—18 animals 1 to 3 hours after sustaining 30% second degree burns were subjected to hypothermia for 4 to 24 hours and were then sacrificed at intervals from 2 to 28 days

During anesthesia vital signs were recorded hourly On 8 of the hypo-

* From the Whitehead Department of Surgery Emory University School of Medicine Atlanta Georgia Supported by the Hartford Foundation

thermic controls liver biopsies and determinations of hematocrit white cell counts with differential serum electrolytes serum proteins non protein nitrogen and bromsulphalein retention were made *prior to hypothermia* and every 8 hours thereafter for 24 hours The remainder had determinations of hematocrit blood volume serum electrolytes and urinary excretion of water electrolytes and 17 ketosteroids at appropriate intervals preburn postburn and/or prehypothermia and at 8 hour intervals thereafter for 24 to 36 hours Weight and status of the burns were recorded at the beginning of the experiment and weekly until sacrifice Gross necropsies were performed at death or sacrifice

After a fast of 12 hours the dogs were anesthetized with nembutal shaved weighed and their body surface area calculated Base line blood determinations were drawn from the inferior vena cava via a femoral cutdown and the bladder was catheterized On the burn controls and experimentals 30% second degree burns were inflicted by an Evans iron [1] After burning repeat base line studies were drawn Immediately in the hypothermia controls and 1 to 3 hours postburn in the burn experimentals hypothermia was induced by means of a cold air isolation box [2] or an ice bath Blood samples were again drawn and every 8 hours thereafter Body temperatures were kept at 28 to 30°C until termination of hypothermia while anesthesia was maintained by small doses of nembutal Five hundred cc 5% glucose in saline and 500 cc 5% glucose in water were administered intravenously during each 12 hour period On warming each hypothermic dog was given 15 cc whole blood per kg of body weight Ten burned animals were given 1 cc. whole blood for every 5 cm. burned Daily 300 000 units penicillin and 0 5 gm streptomycin were administered intramuscularly for 2 weeks Infected burns were treated with topical bacitracin

Urine samples were taken every 8 hours and the volume output recorded All blood and urine determinations were on a standard laboratory basis Blood volumes were determined by use of radioactive chromate tagged red blood cells from which values plasma volume and red cell mass could be calculated by use of the corresponding hematocrits

RESULTS

Hypothermia could be achieved by inhalation and exposure to cold air in two thirds the time required by ice bath immersion Pulse and respiratory rates were directly proportional to temperature regardless of whether burned and/or cooled Below 28 4°C difficulty was experienced with apnea and this was the contributing cause of death in the majority of cases During the period of hypothermia there was marked deceleration of fluid accumulation in the burn sites but on return to normothermia the expected edema appeared

In the hypothermia controls white cell counts fell to one half the precooling levels mainly due to a neutropenia The eosinophil counts increased by three and one half times Hematocrits increased 10% in the first 8 hours and 4% with each additional 8 hours of hypothermia The burned animals showed an even greater increase (15% and 6% respectively)

Blood volumes fell in all groups almost entirely secondary to a concomitant reduction in plasma volume The hypothermia controls had a reduction in blood volume of 20% during the first 8 hours with an additional 5% diminu

thermia had 22% as clean granulation in addition to the 5% area infected. By 4 weeks, all burn sites were clean and epithelialized. Also at 4 weeks, weight loss of the burned experimentals was approximately two-thirds that of the burned controls.

Necropsies revealed only an occasional focal hemorrhage in the small bowel, kidney, pancreas, and adrenal. A few animals had small ulcers in the jejunum. Microscopic liver sections showed evidence of passive congestion. Bronchopneumonia was noted in 2 animals that died late in the follow-up period. Seven animals were sacrificed prematurely, and 3 cooled animals died of apnea. Overall, there were no significant differences between burned controls and experimentals.

COMMENTS

These studies definitely substantiate the fact that hypothermia is a situation of stress, despite its reduction of metabolic rate. Prolonged hypothermia is undesirable, since difficulties arise with respect to pulmonary ventilation, evaluation of blood and plasma loss, maintenance of electrolyte balance, and resistance to infection. These are reflections of an inhibition to the respiratory center, alteration in renal tubular function, and depression of the reticulo-endothelial system.

However, when instituted for only a few hours, hypothermia can be useful. A brief period of hypothermia during the initial treatment of the severely burned dog is well tolerated and such a procedure may be of considerable aid in the human. The metabolic rate is definitely lowered. Through peripheral vasoconstriction, the loss of colloid, electrolyte, and fluid into the burned areas is diminished while urinary excretion of water and especially potassium is augmented; if hypothermia is not prolonged, these aid considerably the control of fluid and electrolyte balances. During the first 4 to 6 hours of hypothermia, there is only moderate alteration of blood volume relationships so that adequate time is available for colloid replacement. The severity of burn is certainly not increased and these studies strongly suggest that it is actually diminished so long as hypothermia is not prolonged.

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EXPERIMENTAL STUDIES ON ACUTE RENAL TUBULAR DEGENERATION FOLLOWING CRUSH INJURY *

SAMUEL R POWERS JR ANTONIO BOBA NOBUYUKI SHIOYA
AND ARTHUR A STEIN

Acute renal insufficiency is commonly associated with severe trauma especially when large muscle masses are severely damaged The present study was undertaken with the aim of studying the renal hemodynamics following graded mallet trauma to the legs of dogs Previous studies[1] have demonstrated that cross clamping of the aorta just below the level of the renal arteries caused a profound fall in renal blood flow without systemic hypotension and was associated with renal tubular degeneration These observations prompted a study of changes in renal vascular resistance without systemic hypotension with graded muscle trauma as the etiologic agent

METHOD

Mongrel dogs anesthetized with pentobarbital anesthesia were subjected to 350 to 600 blows with a light leather mallet to one leg It was found by trial and error that this degree of trauma was sufficient to induce changes in renal blood flow with little if any change in systemic blood pressure

Direct renal blood flow was obtained from a T cannula placed in the left renal vein The renal vein was first exposed through a transperitoneal approach using a flank incision This vein was carefully lifted out of the renal pedicle without excessive mobilization of the kidney and without disturbing either the renal artery or its surrounding nerve supply If either of these structures are traumatized a partial renal denervation will result and the effect of trauma will be altered In order to insert the T cannula it was necessary to temporarily occlude the renal artery by placing a non crushing vascular clamp across the remaining undissected renal pedicle The period of total renal ischemia did not exceed 3 to 4 minutes With this cannula in place renal venous blood could pass either directly into the vena cava (pressure drop due to the cannula 3 mm H_2O) or by occlusion of the caval side it could be diverted into a graduated cylinder and measured All blood collected for flow measurements was returned to the animal through a catheter in the jugular vein Samples of renal arterial and venous blood were separately drawn and analyzed for oxygen content on a Beckmann DU spectrophotometer Arterial pressure was continuously recorded from a catheter passed down the left carotid artery to the region of the renal artery orifices Twenty five mg of heparin had been instilled into the side arm of the T cannula to prevent clot formation and interference with renal venous return

RESULTS

Group 1 In 6 control animals trauma was withheld but the experiment was otherwise identical with the other groups Serial blood flow and oxygen consumption studies were carried out from 2 to 4 hours The average renal blood flow was 3 7 cc /min /gm of kidney None of these dogs showed a sig

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nificant change in renal hemodynamics (variation $\pm 10\%$). At the conclusion of the experiment the cannulated kidney was removed and the wound closed. Seventy two hours later the animal was sacrificed and the undisturbed kidney removed for histologic study. All six kidneys were histologically within normal limits

Group 2. Twenty-four animals were subjected to 300 to 600 blows with a light leather mallet to one leg. Eighteen of these animals survived and were suitable for histologic examination of the kidneys. Eleven of the eighteen survivors showed microscopic changes consisting of extensive, although patchy, tubular degeneration

Sections of all kidneys removed at the time of surgery and at sacrifice 72 hours after the experiment were prepared. All organs were coded and a serial numerical system unknown to the examiner was set up The slides from each animal were submitted "blind" to the pathologist for study.

It was apparent from past experience that clinically valid interpretations of the experimental kidney must be made on gross histologic findings and must not be based on localized cytologic disturbances; therefore, tubular degeneration of all kidneys was classified as plus, plus/minus, or minus. Following severe trauma, a majority of animals developed an extensive although patchy hydropic degeneration of tubular epithelium. Although different segments of the nephron were involved, maximum damage appeared to involve the distal tubule The epithelial cells were markedly swollen, contained multiple cytoplasmic vacuoles, and showed displacement of their nuclei. Some cells were separated from the basement membrane. These tubules contained albuminous fluid and only rarely hemoglobin casts. There was no inflammatory cell response

Although severe trauma was not associated with a specific histologic lesion which can be readily quantitated, extensive hydropic tubular damage was found in the majority of animals Degenerative changes of the kidney were not observed in similarly traumatized dogs which had either received Arfonad or had renal denervation

Ten of the 24 animals had renal hemodynamic studies as described under *Method* In each case, control measurements of renal blood flow, blood pressure, and renal oxygen extraction were carried out as in Group 1 and the animal was then subjected to trauma These measurements were repeated at 15 minute intervals for up to 2 hours, depending upon the condition of the animal From these data, renal vascular resistance and renal oxygen consumption were calculated Figure 1 shows the changes in renal vascular resistance in this set of experiments With little associated change in blood pressure, a significant fall in renal blood flow indicated an increase in renal vascular resistance In most of the animals, the oxygen extraction in creased to an extent that was roughly proportional to the decrease in flow so that the calculated oxygen consumption of the kidneys was essentially unchanged.

Group 3. In three animals the left kidney was denervated prior to trauma This was accomplished by complete transection of the renal pedicle and rapid reanastomosis of the renal artery and vein over cannulae. Observations were carried out in an identical manner to Group II and showed no change in renal vascular resistance up to four hours after trauma In one animal with more severe trauma, the systemic blood pressure fell and there was an asso

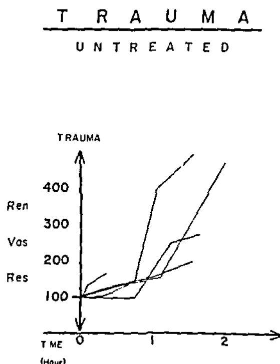


Fig 1 Proportional changes in renal vascular resistance following mallet trauma in 6 animals The control value in each case was arbitrarily taken as 100 Blood pressure in all animals remained within 10% of control values

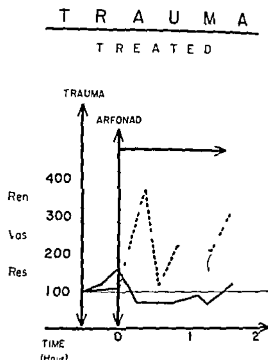


Fig 2 Changes in renal vascular resistance resulting from the administration of Arfonad after the completion of trauma The solid line was calculated from an animal in which the blood pressure was kept near 85 mm Hg The dotted line was calculated from an animal with a blood pressure which fluctuated between 30 and 60 mm Hg Extreme hypotension apparently produces an effective increase in renal vascular resistance

ciated fall in renal blood flow, but without any significant change in the calculated renal vascular resistance

Group 4. Six animals were treated with Arfonad, a ganglionic blocking agent, following the completion of mallet trauma The rate of drug administration was adjusted so as to achieve a predetermined mean aortic blood pressure Various degrees of hypotension were used in an attempt to determine the optimal level for protection of the kidneys Figure 2 shows the results of two such experiments with moderate and profound hypotension One animal with very low blood pressure had a marked increase in renal vascular resistance and this was the only animal of the Arfonad treated group which showed acute renal tubular degeneration The other five animals showed either no change in renal vascular resistance or a moderate increase An associated observation was an increased oxygen extraction by the kidney following ganglionic blockade in all cases It appears that ganglionic blockade provides some measure of protection to the kidney as long as the mean aortic pressure is kept above 85 mm Hg

SUMMARY AND CONCLUSIONS

Mallet trauma to one leg of a dog will produce hemodynamic and histologic evidence of renal ischemia in the absence of hypotension in a significant number of animals The pathologic changes produced are those of severe hydropic

degeneration of tubular epithelium The tubular damage is at least in part due to a greatly increased renal vascular resistance, mediated through the autonomic nerve supply of the kidney. Surgical denervation or pharmacho logic blockade modifies this response and offers some protection of the renal tubules

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LACTIC DEHYDROGENASE ACTIVITY OF DOG PLASMA AND URINE FOLLOWING RENAL INJURY *

MURRAY M. BITT, JEROME D SKAGGS, GLORIA JOHNSTON, AND FALLS B HERSHEY

Following tissue injury, intracellular enzymes appear in the circulation and their identification is useful in the diagnosis of certain disease states.[1,2] This study shows the effects of renal injury in the dog on the plasma and urinary lactic dehydrogenase (LDH) LDH is selected because of its ease of estimation in the blood and urine, and because it is abundant in dog kidney. LDH is not a specific renal enzyme and is present in other dog tissues.

METHOD

Two types of renal injury were investigated—infarction and ischemia. All experiments were performed on anesthetized dogs through a midline abdominal incision with mobilization of the kidneys, dissection of the renal pedicles, and suturing of the renal capsule to the perinephric tissue prior to closure to avoid torsion of the renal vein All animals were sacrificed 7 to 10 days later and the kidneys examined pathologically

Peripheral venous blood samples were heparinized, centrifuged, and the plasma collected The method of LDH estimation is that of Wacker, Vallee,[3] and Calman [4] Plasma samples added to stock reagents containing lactic acid, and diphosphopyridine nucleotide (DPN) at 33°C. form pyruvic acid and DPNH The DPNH is measured on the coenzometer, a calibrated instrument with a light source of fixed wave length The change of optical density of DPNH as it is formed is recorded over a time interval of 3 to 5 minutes

LDH activity can be expressed in units/minute/ml of plasma

It was found that urine samples containing LDH could also be measured in a similar fashion Urine samples were also examined for protein, red blood cells, and the pH recorded The presence of protein did not affect the LDH activity of urine Hydrogen ion concentration in the ranges obtained in our urine samples did not appear to affect the activity of LDH Blood detected

* From the Department of Surgery Washington University School of Medicine and the Cochran Veterans Administration Hospital St Louis Supported in part by grants from the Institute for Medical Education and Research The Harry Freund Memorial Foundation of St Louis, Missouri and the U S Public Health Service RG 4192 (C3)

chemically and microscopically does appear to affect the urine LDH especially in gross amounts The amount of blood present in urine to cause false elevation of LDH by the above method of measurement is recorded as +++ Lesser amounts of blood recorded as + or ++ do not cause a measurable increase of LDH

Normal LDH Values. Normal values for plasma LDH levels in 46 dogs showed a range of 0 to 100 units with a mean of 46 units Urine LDH levels in 20 dogs showed a range of 10 to 150 units with a mean of 37 units The means and level for two standard deviations are plotted on the charts

RESULTS

Thirty four dogs were used in this study, including 11 control animals

Renal infarcts were produced in 12 dogs by selective interruption of the branches of one renal artery, comprising approximately 75% of the arterial supply to that kidney There was a sustained marked elevation of plasma LDH in all dogs, persisting for at least 48 hours followed by a gradual return to normal levels Figure 1 illustrates this effect in 6 of those dogs The urine LDH was followed in these 6 dogs and although an occasional slight immediate elevation was noted the general trend was toward a slowly progressive rise in activity after 48 hours However, this rise was associated with the appearance of hematuria All dogs were sacrificed and these kidneys showed classical cortical infarcts involving one half to two thirds of the cortical area

Renal ischemia was produced in 11 dogs by occluding both renal arteries for 1½ to 2 hours in 9 dogs (Group A), and 3 hours in 2 dogs (Group B) An immediate elevation of plasma LDH was noted in 5 dogs of Group A followed by a rapid fall in 12 to 24 hours The other 4 dogs in this group showed no significant elevation However, Group B dogs showed profound elevations of LDH falling to normal in 12 to 24 hours The 2 dogs in this group showed marked lethargy postoperatively, producing small amounts of urine, and 1 dog died 72 hours later of renal failure It is interesting that at the time of death the plasma LDH had fallen to normal levels Figure 2 illustrates these findings in 3 dogs in Group A and the 2 dogs in Group B *Figure 3 shows the urine LDH levels in the same dogs* Similar immediate quantitative relationship between the degree of renal ischemia and the LDH level in the urine are present, the 3 hour occlusion dogs producing more enzyme at least initially However, the later appearance of blood and pus in the urine after 48 hours tends to invalidate the high levels recorded after 48 hours

Autopsy findings showed severe changes after 3 hours of occlusion There

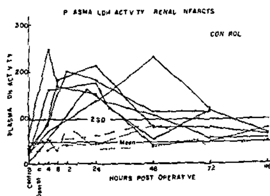


Fig 1 Plasma LDH—Renal infarction

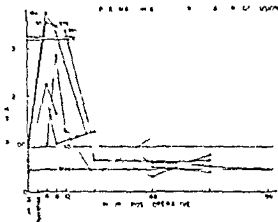


Fig 2 Plasma LDH—Renal artery occlusion

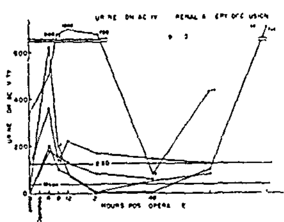


Fig 3 Urine LDH—Renal artery occlusion

were areas of complete cortical necrosis interspersed with areas showing severe tubular damage After 1½ to 2 hours of occlusion the kidneys showed mainly degenerative changes of the tubules with edema congestion and little if any necrosis

Control experiments including mobilization of the kidneys in 7 dogs unilateral nephrectomies in 3 dogs and opening and closing a laporatomy wound in 1 dog showed no postoperative elevation of plasma or urine LDH Three of these dogs are illustrated in Figure 1

DISCUSSION

Renal ischemia produces immediate plasma LDH elevation which bears a relationship to the severity of the cortical damage produced as judged pathologically The factor of necrosis appears to influence the output of LDH This release of enzyme in the circulation is similarly reflected in the urine However after 12 to 24 hours enzyme ceases to be released from the damaged kidney irrespective of the clinical course of the dog and the recuperative *power of the kidney Renal infarction produces more sustained elevations of* plasma LDH lasting for 48 hours before returning to normal Here urine levels of LDH show little tendency to an immediate postoperative rise but a gradual rise in activity after 24 hours probably reflects the presence of increasing amounts of blood

CONCLUSIONS

1 Renal infarction and ischemia cause significant elevations of plasma LDH in the dog

2 The magnitude of these plasma levels are roughly proportional to the severity of renal injury and are higher when there is necrosis

3 The urine levels of LDH following renal ischemia roughly parallel the plasma levels

4 Gross hematuria increases LDH levels in the urine

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EXPERIMENTAL STUDY OF INTRAVENOUS FAT EMULSIONS *

MOHAMMAD ATIK, JOSHUA C WERNER AND ISIDORE COHN JR

Serious adverse clinical effects of long term intravenous fat administration have been reported recently by a number of investigators [1 2] Since some of these untoward reactions have not been adequately explained this experimental work was undertaken to study the physiopathology concerned in an attempt to uncover some of the underlying mechanisms

METHOD

Eight normal dogs weighing 11 to 17 kg were given intravenous fat emulsion (supplied by the Upjohn Company as Lipomul I V) 5 times weekly Monday through Friday until such time as the dogs died

A plastic tube was inserted into the external jugular vein of the animals and brought out subcutaneously to the back of the neck so that the animals could be given the fat preparation while sitting or walking around the cage at will No other operation was performed upon them The dogs were allowed the usual kennel diet and water *ad lib* Each animal received 600 cc of Lipomul each day over a 3 hour period On the basis of original weight the daily dose varied from 35 to 54 5 ml /kg of body weight The amount of fat emulsion which the animals received varied from 3 to 16 liters between the day of the first infusion and the time of the animals death (Figure 1) The animals were observed daily and blood samples were drawn at intervals for counts and studies of blood chemistry Special serum lipid and protein analyses were conducted by the Southern Regional Research Laboratory [3] All dogs were autopsied at death Histologic sections were obtained for study by our group as well as by the Army Medical Nutrition Laboratory in Denver

RESULTS

The animals lost considerable weight became anorexic vomited at intervals terminally became progressively lethargic and died between 7 and 56 days after the first infusion Almost all developed infection at the cut down site There was a progressive drop in hemoglobin red count and hematocrit An elevation of the white count was noted in all animals but this was not progressive throughout the study

Laboratory studies revealed no significant changes in blood sugar direct and total bilirubin prothrombin time coagulation time or in platelet counts

* From the Department of Surgery Louisiana State University School of Medicine New Orleans Supported by the Research and Development Division Office of the Surgeon General Department of the Army under Contract No DA 49 007 MD 8/9

All serum electrophoretic studies were conducted by Ruth R Benerito Ph D and Katherine M Talluto B S of the Southern Utilization Research and Development Division U S Department of Agriculture New Orleans

Fig. 1. Duration and amount of intravenous fat administration in dogs. Arrow points to the animals' death

There was a terminal increase in blood urea nitrogen in one animal but all others remained within the normal range. BSP retention was within normal limits in 3 dogs but 2 dogs showed increases to 10% and 26% dye retention prior to death.

Certain changes were noted in the fat clearance and the serum protein analysis. As the infusions of fat continued, all animals displayed lipemia. This process was noted to be cumulative. During the first week the animals were able to clear the serum of fat by the morning following the infusion. During the middle of the second week, they showed lipemia on the morning following an infusion. As the weeks progressed, even the 72 hours of infusion-free period during the weekend failed to provide sufficient time for the animals to clear the serum of fat.

Two dogs which had a 2 week interruption of intravenous fat administration were able to clear the serum of fat during that time but with the resumption of fat infusion, persistent lipemia appeared after fewer infusions than it did in previously normal dogs. Thus as the period of fat administration continued, the ability to clear the serum of fat became progressively impaired until the animals were no longer able to clear the serum of fat at all. Even two weeks of an infusion-free period had not permitted the fat clearing mechanism to return to normal.

In serum protein analysis the following interesting changes were noted: (Fig 2)

1. The total serum protein determined by the Kjeldahl and Biuret method dropped to approximately 10% of the control value.

2. The serum albumin content, as determined by the salt fractionation method, showed a slight drop of the same order of magnitude as the serum protein.

Fig 2. *Serum proteins Dog 5116. Note the divergence of serum albumin determined by the two methods of salt fractionation and electrophoresis*

3 When serum albumin was determined electrophoretically there was an initial comparable decrease followed by a progressive drop to 10 to 50% of the control value

4 The serum albumin content determined by the two methods diverged as the number of infusions increased

5 The serum gamma globulin showed a variable change with initial drop and subsequent rise Those dogs which survived only 5 to 10 infusions revealed a drastic drop of 80 to 90% prior to death

6 The alpha and beta globulin separated poorly, with most of these fractions having an electrophoreogram akin to that of beta lipoprotein

7 There was a correlation between the clinical condition of the dogs and the drop in serum proteins determined electrophoretically

At autopsy there was bloody fluid in the pleural cavity in 5 dogs and in the peritoneal cavity in 6 In none of them was there an adequate explanation for the presence of these bloody effusions There was purulent fluid in the pleural cavity in 2 animals The organs were pale and greasy in 6 animals and essentially normal in the other 2 In the last 2 dogs there was gastrointestinal ulceration There was consolidation, edema, or atelectasis of the lungs in 6 animals The precise cause of death was not apparent from the gross autopsy findings in 5 animals Death was attributed to rupture of an abscess of the neck into the pleural cavity in one, an abscess just above the apex of the lung in one, and acute duodenal ulceration with hemorrhage in one The explanation for each of these findings is not known since intravenous fat therapy was the only type of experimental procedure to which these animals were subjected

Microscopic sections revealed pneumonitis and pulmonary congestion in all of the animals and pulmonary edema in three The liver sections showed areas of focal necrosis, varying amounts of passive congestion and fatty infiltration and pigmentation Two of the dogs had probable degeneration and fatty infiltration of the renal tubules The spleen appeared congested and had pigmentation in all of the dogs The pancreas adrenals, and bone marrow showed no significant abnormalities except for the pigment deposits in the bone marrow Reports from the group in Denver indicate that the "so called intravenous fat emulsion pigment was found in all eight animals [4]

DISCUSSION

Much investigative work has been centered on the preparation of a safe intravenous fat emulsion to provide sufficient calories for patients in whom oral feeding is contraindicated or not feasible for a prolonged period While the caloric requirements have been met by certain preparations, a number of acute and chronic reactions have prohibited their clinical use Lipomul at first seemed promising in this respect, and its use for short periods did not result in some of the acute toxicity that had been observed with earlier preparations However reports of severe reactions began to appear which stimulated our interest in this study

The 8 animals in this study were given higher doses of Lipomul than is generally recommended with the full realization that this might enhance the toxicity and make these deleterious effects amenable to study The progressive weight loss, unusual susceptibility to infection and death of all of these animals after administration of intravenous fat emulsion in this dosage

range, provided an opportunity to study these untoward effects. The dogs developed a progressive anemia which could not be entirely explained in the absence of evidence of a disturbed erythropoietic mechanism, a significant hemolytic process, appreciable gastrointestinal bleeding, or hemodilution, which was out of proportion to what one might expect from the amount of blood withdrawn for study. The leukocytosis is understandable in view of repeated and terminal infection in most of the animals. The cause of gastrointestinal ulceration remains obscure.

The progressive lipemia and the dissociation between the serum protein values obtained by electrophoresis and salt fractionation, strongly suggest the excessive formation of a protein lipid complex and some impairment of the fat clearing mechanism. This may mean that while the total body serum proteins remain unchanged, the free serum proteins may drop on account of their forming a complex with the excessive circulating lipids. Such a complex may make the proteins unavailable for their usual functions and help to explain the weight loss, anemia, and the lowered resistance to infection.

These preliminary observations provide a plausible hypothesis for the chronic toxicity of intravenous fat emulsion based upon a deranged fat clearance and perhaps a disturbed protein metabolism. It is to be hoped that a safer fat emulsion can be prepared on the basis of these studies.

SUMMARY AND CONCLUSION

The untoward effects of long term intravenous fat emulsion therapy were studied in 8 dogs. The dogs were given 600 cc. of Lipomul intravenously daily in addition to a normal diet. The animals showed progressive anorexia, weight loss, unusual susceptibility to infection, sustained lipemia, anemia and leukocytosis. All died within one to six weeks of this treatment.

Analysis of serum proteins revealed an apparent progressive drop in serum albumin and variable decrease in serum gamma globulin as determined electrophoretically. This apparent drop in serum proteins could be correlated with the clinical condition of the animals. The alpha and beta globulins could not be separated with ease and showed an electrophoretic pattern similar to that of lipoproteins

It is suggested that the impaired fat clearing mechanism and the disturbed protein metabolism are perhaps based on the "binding" of the serum proteins to the lipids which may make them unavailable for some physiologic processes.

It may be concluded that the long term administration of intravenous fat emulsion over 35 ml /kg. of body weight is not safe

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A STUDY OF DIFFERENCES IN FAT ABSORPTION FOLLOWING ORAL AND JEJUNAL ADMINISTERED I131 LABELED FAT *

WALTER G GOBBEL, JR, AND HARRISON H SHOULDERS, JR.

One of the most undesirable sequelae which may follow subtotal or total gastrectomy is the difficulty in maintaining adequate nutrition Metabolic studies by Wollaeger,[8] Shingleton,[6,7] and others[4] showed higher fat losses in patients following Billroth II than in Billroth I type resections Everson[2] [illegible]

These experiments were undertaken on normal patients to determine the effect of bypassing the duodenum on fat digestion and absorption

METHOD

In 13 patients without gastrointestinal disease a long Cantor tube with mercury filled bag was passed into the stomach and allowed to work its way into the duodenum and jejunum Five additional patients, in whom various types of gastric resection had been carried out and in whom indwelling jejunostomy tubes were placed during the operative procedure, were studied in the second postoperative week and at intervals thereafter A test meal consisting of 37 cc. of peanut oil, 37 cc of water, 1 cc. of tween 80, and 50 microcuries of I131 labeled Triolein was prepared after the method of Shingleton and administered either orally, by way of the tube directly into the duodenum, or by way of the tube directly into the jejunum

Venous blood samples were drawn at 2 3, 4, 5, and 6 hours following the test meal and the total radioactivity in the blood was calculated and expressed as a percentage of the total ingested meal All stool samples were collected for a period of 48 hours following ingestion of this meal The amount of radioactivity excreted was measured and expressed as a percentage of the total ingested test meal Thirty two blood level determinations and 18 stool excretion levels were performed in these 18 patients In those patients in whom the procedure was repeated a minimum of 4 days was allowed to elapse between procedures and a baseline of blood radioactivity was determined prior to administering the test meal In a number of these patients the blood absorption and the stool excretion levels were studied after both oral and jejunal feedings so that a comparison could be made of the two routes of administration in the same individual

RESULTS

Following oral administration of the test meal in 8 patients the mean level at 2, 3, 4, 5, and 6 hours after ingestion was 10 8 13 9, 15 0, 14 3 and 14 7% respectively (Figure 1) In 4 patients in whom the test meal was administered by way of an indwelling duodenal tube the mean levels were 12 5, 14 6, 14 6

* From the Surgical Service and Radioisotope Service, Thayer Veterans Administration Hospital and the Department of Surgery, Vanderbilt University School of Medicine Nashville

15 0 and 12 7% Thus, the rates of digestion and absorption after oral and duodenal administration were approximately the same

Following jejunal administration the levels were 3 2, 4 1, 3 8, 3 8, and 4 1% respectively for the 2, 3, 4, 5 and 6 hour observations in a total of 20 patient determinations

The average fecal excretion in 18 hours following administration of the test meal was 3 8% in 3 patients following oral administration, 1 6% in 1 patients following duodenal administration, and 31 8% in 11 patients following jejunal administration (Figure 2) There was no difference in rate of absorption or in fecal excretion of radioactivity between normal patients and post gastrectomy patients when the test meal was administered directly into the jejunum

DISCUSSION

Previous studies have shown that the incidence of steatorrhea and malnutrition following any gastric surgery in which the duodenum is bypassed is greater than in a procedure in which the duodenum is not bypassed (Billroth I) However, Zollinger and Williams[9] believe that the weight loss in postgastrectomized patients is related to the amount of stomach removed, while others believe that deficient caloric intake is of paramount importance Although an inadequate reservoir at times may be important, an interesting study by Shingleton[7] showed that in 21 patients who had a vagotomy with gastroenterostomy, with the entire stomach remaining as a reservoir, 7 of the patients were under ideal weight and the average fecal excretion following the Triolein test meal in these patients was 22%

In 1902 Bayliss and Starling[1] showed that when acid (and later Ivy[3] with meats and fats) comes in contact with the duodenal mucosa it changes a prosecretin to secretin that stimulates the pancreas to secrete its digestive enzymes Secretin also stimulates the secretion of bile In this experiment the lowered fat absorption and increased fecal excretion in patients receiving the test meal directly into the jejunum would suggest that the stimulation of the duodenum by the test meal is extremely important in liberating fat splitting enzymes for normal fat digestion, for in the patients who received the test meal orally and by way of the duodenum the blood levels and stool losses were within normal limits Although it is well known that succus entericus is secreted by the jejunum and ileum following food contact stimulation, it is apparent from these studies that sufficient fat splitting enzymes are not liberated by this

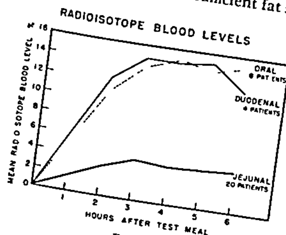


Fig 1

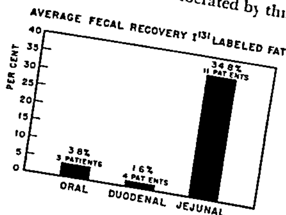


Fig 2

A STUDY OF DIFFERENCES IN FAT ABSORPTION FOLLOWING ORAL AND JEJUNAL ADMINISTERED I^{131} LABELED FAT *

WALTER G GOBBEL, JR, AND HARRISON H SHOULDERS, JR

One of the most undesirable sequelae which may follow subtotal or total gastrectomy is the difficulty in maintaining adequate nutrition Metabolic studies by Wollaeger,[8] Shingleton,[6, 7] and others[4] showed higher fat losses in patients following Billroth II than in Billroth I type resections Everson[2] has extended and confirmed these studies in dogs Plzak and associates[5] have shown in dogs that the stomach exercises no influence *per se* over the digestive processes in the small intestine as regards protein and fat

These experiments were undertaken on normal patients to determine the effect of bypassing the duodenum on fat digestion and absorption

METHOD

In 13 patients without gastrointestinal disease a long Cantor tube with mercury filled bag was passed into the stomach and allowed to work its way into the duodenum and jejunum Five additional patients, in whom various types of gastric resection had been carried out and in whom indwelling jejunostomy tubes were placed during the operative procedure, were studied in the second postoperative week and at intervals thereafter A test meal consisting of 37 cc of peanut oil, 37 cc of water, 1 cc of tween 80, and 50 microcuries of I^{131} labeled Triolein was prepared after the method of Shingleton and administered either orally, by way of the tube directly into the duodenum or by way of the tube directly into the jejunum

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* From the Surgical Service and Radioisotope Service Thayer Veterans Administration Hospital and the Department of Surgery, Vanderbilt University School of Medicine Nashville

15.0 and 12.7%. Thus, the rates of digestion and absorption after oral and duodenal administration were approximately the same.

Following jejunal administration the levels were 3.2, 4.1, 3.8, 3.8, and 4.1% respectively for the 2, 3, 4, 5 and 6 hour observations in a total of 20 patient determinations.

The average fecal excretion in 48 hours following administration of the test meal was 3.8% in 3 patients following oral administration, 1.6% in 4 patients following duodenal administration, and 31.8% in 11 patients following jejunal administration (Figure 2). There was no difference in rate of absorption or in fecal excretion of radioactivity between normal patients and post-gastrectomy patients when the test meal was administered directly into the jejunum.

DISCUSSION

Previous studies have shown that the incidence of steatorrhea and malnutrition following any gastric surgery in which the duodenum is bypassed is greater than in a procedure in which the duodenum is not bypassed (Billroth I). However, Zollinger and Williams[9] believe that the weight loss in post-gastrectomized patients is related to the amount of stomach removed, while others believe that deficient caloric intake is of paramount importance. Although an inadequate reservoir at times may be important, an interesting study by Shingleton[7] showed that in 21 patients who had a vagotomy with gastroenterostomy, with the entire stomach remaining as a reservoir, 7 of the patients were under ideal weight and the average fecal excretion following the Triolein test meal in these patients was 22%.

In 1902 Bayliss and Starling[1] showed that when acid (and later Ivy[3] with meats and fats) comes in contact with the duodenal mucosa it changes a pro secretin to secretin that stimulates the pancreas to secrete its digestive enzymes. Secretin also stimulates the secretion of bile. In this experiment the lowered fat absorption and increased fecal excretion in patients receiving the test meal directly into the jejunum would suggest that the stimulation of the duodenum by the test meal is extremely important in liberating fat splitting enzymes for normal fat digestion, for in the patients who received the test meal orally and by way of the duodenum the blood levels and stool losses were within normal limits. Although it is well known that succus entericus is secreted by the jejunum and ileum following food contact stimulation, it is apparent from these studies that sufficient fat splitting enzymes are not liberated by this

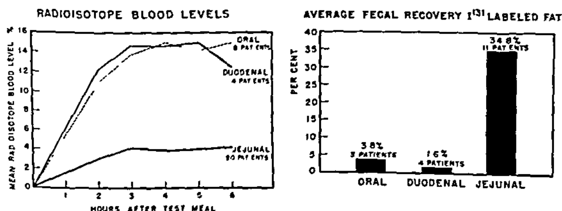


Fig. 1

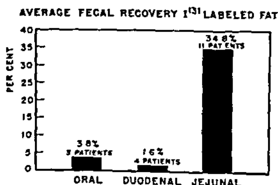


Fig. 2

stimulation alone, and apparently the passage of food through the duodenum is necessary to bring about adequate pancreatic enzyme production for normal fat digestion. The results of this experiment further suggest that gastric lipase and possibly other hormonal factors, liberated by the stomach following stimulation with food, are not necessary in fat digestion and absorption since the patients in which the fat meal was placed directly into the duodenum absorbed an equal amount of radioactivity and excreted an equally small amount of radioactivity in the stool as those who received the meal orally

These findings add further support to those who advocate a reconstitution by a gastroduodenostomy following varying degrees of gastric resection in an effort to maintain the individual in a better nutritional state and in avoiding steatorrhea.

SUMMARY

Fat absorption studies in normal patients without gastrointestinal disease showed that blood levels of radioactivity following jejunal feedings of I^{131} labeled fat are approximately one-fourth of those following oral and duodenal feedings

The total fecal radioactivity excreted in 48 hours following jejunal administration was approximately tenfold greater than in patients following oral and duodenal feedings

It is concluded that duodenal stimulation by the ingested fat is necessary to produce an adequate hormonal enzymatic response to afford normal digestion and absorption.

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THE HEMOLYTIC EFFECTS OF FAT EMULSION *

HARRY H LEVEEN, GERALD FALK AND PRUDENCE GIORDANO

Animals or humans on high fat diets often develop a hemolytic type of anemia This anemia has been attributed to the absorption of fatty acids [1] After a number of infusions of fat emulsion a mild hemolytic type of anemia ensues [2] In order to bypass this and other difficulties inherent in the formation and infusion of fat emulsions Shafiroff and Mulholland [3] tested the hemolytic effects of plasma from donors made lipemic by the ingestion of fat The lipemic plasma was hemolytic when tested against washed red blood cells *in vitro* Since the publication of these papers the nature of the fat clearing process in plasma has become better understood Shore [4] demonstrated that neutral fats undergo lipolysis with the formation of fatty acid and glycerol during the clearing process The liberated fatty acid becomes attached to plasma albumin and migrates with this protein fraction on electrophoresis Surface active properties of fatty acids render them effective hemolytic agents [5] The hemolytic effects of intravenously administered fat emulsions and high fat diets may result during lipemic clearing from the formation of free fatty acids in excess of that which can be bound by serum albumin The present investigation was directed toward testing the validity of this attractive hypothesis

METHOD

A commercially available intravenous fat emulsion† was used as a substrate for *in vitro* experiments

Lipomul I V when tested *in vitro* against heparinized citrated blood without clearing activity failed to initiate hemolysis even after 48 hours of incubation at 37°C This demonstrated that the emulsion itself is not hemolytic with respect to whole blood if clearing does not occur When washed human erythrocytes are suspended in saline and tested against this fat emulsion immediate hemolysis of the red cells occurs This indicates that the plasma proteins assert a protective action against the hemolytic effects of the emulsion itself

Blood samples with enhanced clearing activity were obtained by prior infusion of heparin (10 to 15 mg i v) Citrate or oxylate was used as an anticoagulant *In vitro* clearing was allowed to occur by adding 2 mg of fat in 0 2 cc of diluted emulsion to 1 cc of plasma plus 0 2 cc erythrocytes The blood emulsion mixtures were incubated for varying periods up to 48 hours Hemolysis usually occurred in 8 to 10 hours in rapid clearers (Figure 1) The clearing activity was measured by determining 1) the rate at which the plasma alone would clear the emulsion and 2) by determining the quantity of unesterified fatty acid formed in the blood emulsion mixture No hemolysis was observed when unesterified fatty acid levels remained beneath 10 000 μEq of unesterified fatty acid (58 determinations) Hemolysis was observed in every instance (26 determinations) when the unesterified fatty acid level was above 11 000 μEq of unesterified fatty acid

† Lipomul I V was made available by Upjohn & Co

* From the Departments of Surgery Brooklyn VA Hospital and State University of N Y Downstate Medical Center Supported in part by Grant No A 1632(R1) from the U S Public Health Service

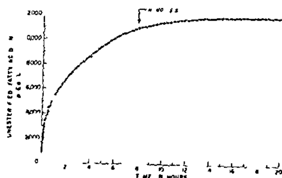


Fig 1 The release of free fatty acid during clearing of the emulsion is illustrated If red cells are present during clearing hemolysis will occur at levels of 11 000 μEq/l of unesterified fatty acid

RESULTS

These results suggested that the maximum binding capacity of the serum proteins for unesterified fatty acid was 11,000 μEq/L In another experiment additional serum albumin was added to plasma and blood cells to ascertain whether it would protect against hemolysis if unesterified fatty acid levels were 11,000 μEq/L The amount of plasma albumin in whole blood was roughly doubled by the addition of purified serum albumin Fat emulsion was added and the mixtures were again incubated and the unesterified fatty acid levels were measured In no instance was hemolysis observed even when unesterified fatty acid levels were as high as 18,000 μEq/L We were unable to measure the quantitative protection, but it was considerable *(Figure 2)*

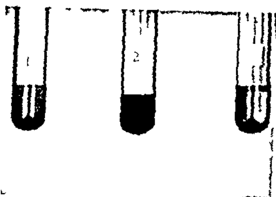


Fig 2 Illustrates the protection against hemolysis which is afforded by serum albumin In tube 1 1600 μEq/L of unesterified fatty acid have been released by plasma There is no hemolysis In tube 2 12000 μEq/L of fatty acid have been released by postheparin clearing Hemolysis is evident In tube 3 the postheparin clearing has continued to a level of 15 000 μEq/L of fatty acid but no hemolysis has taken place because the serum albumin concentration has been doubled

SUMMARY AND CONCLUSIONS

Although it is hazardous to draw conclusions about *in vivo* processes from *in vitro* experiments, the data suggest that the hemolytic effects of fat emulsion might result from an excessive amount of free fatty acid formed during the clearing process It has not yet been demonstrated that intravenous fat emulsion clears from the plasma They may be removed *in vitro* as neutral fats by the clearing mechanism

When free fatty acid levels rise beyond the binding capacity of serum albumin, the fatty acids attach themselves to and cause lysis of the formed elements in the blood

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THE EFFECT OF LONG TERM ADMINISTRATION OF INTRAVENOUS FAT ON LIVER FUNCTION AND THE COAGULATION MECHANISM *

CURTIS P ARTZ J HAROLD CONN AND WARREN N BELL

During the past two years an intravenous fat emulsion† has been under intensive clinical study [1, 2] It has been found to be safe and effective in limited amounts After long term administration of large quantities it was noted that some patients became jaundiced and others bled spontaneously

A study was designed to ascertain the effects of the administration of large quantities of this intravenous fat emulsion in patients by carrying out a battery of liver function tests and coagulograms at regular intervals during therapy

METHOD

Most of the patients selected had debilitating illnesses such as chronic malignancy or chronic brain syndrome During the course of the intravenous fat infusions the patients received protein and calories by the oral route Twenty two patients received a total of 431 units The minimum dose in this study group was 10 units Eleven of the patients who received 14 or more units (600 cc) form the basis of this report

RESULT

The battery of liver function studies included bromsulphalein, cephalin flocculation bilirubin thymol turbidity total protein and A/G ratio and alkaline phosphatase The coagulogram included hematocrit, clotting time, prothrombin time platelet determination prothrombin consumption time plasma hemoglobin fibrinogen titration protamine titration, and thromboplastin generation Daily optical density determinations on the serum did not show any correlation between increased lipemia and dysfunction of the liver and coagulation mechanism

The positive findings of these studies are found in Table 1

Eleven patients received between 14 and 42 units of intravenous fat emulsion In most instances 2 units were administered over a 6 hour period each day In some patients there were a few days during the study in which no fat was given but in general the administration was on consecutive days

Change in Hematocrit In every instance there was a fall in hematocrit during the administration of the fat emulsion This varied from very major decreases in hematocrit to only moderate changes

Plasma Hemoglobin In several instances the plasma hemoglobin was found to be appreciably elevated In one patient red cell survival studies were carried out and the life of the red blood cell was considerably shortened during the period of administration of the intravenous fat emulsion

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† Lipomul I V (600 cc. of 15% cottonseed oil emulsion—960 calories) supplied by the

University of Mississippi
Mississippi and with the
liam Tillman Supported
in part by grants from the Research and Development Division Office of the Surgeon General Department of the Army Contract No DA 49 007 MD-627 and the Upjohn Company Kalamazoo Michigan

Table 1 Effect of Long-term Administration of I V. Fat. Summary of Pertinent Laboratory Data on Patients after Long Term Administration of I.V. Fat

PATIENT AGE AND NO	DIAGNOSIS	UNITS OF FAT	PERIOD OF INFUSION (DAYS)	CHANGE IN HCT (VOL %)	PLASMA HGB (MG %)	HIGHEST BSP (% RETENTION)	HIGHEST BILIRUBIN (MG %)	HIGHEST THYMOL TURBIDITY (UNITS)	PROTHROMBIN TIME (ONE STAGE)	CLOTTING TIME (MINUTES)	PROTHROMBIN CONSUMPTION (SECONDS)	PLATELET COUNT	CLINICAL BLEEDING EPISODES
BF–74 1	Ca–larynx	42	21	33 19	—	14	0 6	1 1	prolonged	25	normal	normal	Hemoptysis, tarry stools after 42 units
WG–66 2	Ca–lung	40	23	40 32	15	19	0 7	5 0	normal	15	16 5	normal	None
HM–58 3	Osteogenic sarcoma	37	27	35 22	13	38	1 5	3 6	normal	15	22 0	normal	None
BJD–64 4	Chronic brain syndrome	32	19	40 32	200	35	8 1	5 6	prolonged	46	14 9	normal	Bled from skin, hematemesis
AW–64 5	Ca–esophagus	30†	15	39 32	—	43	9 8	—	normal	12	23 5	normal	None
DJB–63 6	Chronic brain syndrome	27	15	33 30	17	15	0 8	1 0	normal	7	normal	normal	None
AB–65 7	Ca–larynx	27	14	40-19	4 2	33	3 0	5 0	prolonged	32	21 6	normal	None
RHA–72 8	Chronic brain syndrome	21††	12	34 23	46	26	4 8	11 2	prolonged	23	26	normal	None
JP 9	Ca–lung	18	9	42 36	normal	18	0 1	—	normal	9	20	normal	None
AM–73 10	Ca–esophagus	17	9	44 33	—	14	2.5	1 4	prolonged	19	normal	58,000	Bleeding from intestine
EL–40 11	Ca–stomach	14	8	38 29	normal	10	1 2	1 0	prolonged	12	normal	normal	None

† Had received 26 units 3 months earlier
†† Greatest abnormalities 1 week after fat

Bromsulphalein Retention In every instance there was an increased retention of bromsulphalein This usually occurred after the administration of 10 or 12 units of fat The exact quantitative changes that occurred in the bromsulphalein retention were hard to interpret as the test is affected by many factors However increased retention was a very consistent finding After cessation of infusion the bromsulphalein test returned to normal

Serum Bilirubin In two instances there was obvious clinical jaundice In three other instances there was an elevation of the bilirubin which persisted during the infusion of fat This appears to be a reversible change as it returns to normal after cessation of fat therapy In Patient 5 twenty six units of fat were given with the development of rather severe jaundice Therapy was stopped and the patient s BSP and bilirubin returned to normal Three months later the patient again received 30 units of intravenous fat emulsion over a 15 day period The BSP rose to 13% retention and bilirubin to 9 8 These returned to normal after cessation of therapy

Thymol Turbidity, Cephalin Flocculation, Total Protein and A/G Ratio The thymol turbidity was increased in about half the patients The cephalin flocculation was negative in almost all cases and there was no consistent change in the total protein or A/G ratio

Prothrombin Time The one stage prothrombin time was performed at 4-day intervals during the course of fat infusion This was normal in 5 of the patients and significantly prolonged in 6

Clotting Time The Lee and White coagulation time was prolonged in 5 patients In 3 of these patients clinical manifestations of bleeding occurred

Prothrombin Consumption Time In 7 patients the prothrombin consumption time was diminished to less than 30 seconds during the course of therapy This was one of the most consistent positive coagulogram findings in the entire series

Platelet Count Platelet counts remained normal in all but patient 10 who had an appreciable fall in platelets to 58 000 At this time it was noted that he was bleeding from his lower gastrointestinal tract Intravenous fat emulsion was discontinued for a period of 2 days the platelets returned to normal and additional fat was then given without any bleeding difficulties

Fibrinogen Titration, Protamine Titration, and Thromboplastin Generation There were no appreciable changes in fibrinogen and protamine titrations and thromboplastin generation

Bleeding Episodes Three patients experienced appreciable bleeding after the infusion of the intravenous fat emulsions Patient 1 had hemoptysis and tarry stools after 42 units He experienced a sudden hemorrhage from his larynx and expired It may have been that the hemorrhage followed erosion of a major vessel His clotting and prothrombin times were prolonged

Patient 4 received 32 units over a period of 19 days He bled from several scratches in the skin and had a marked bout of hematemesis The one stage prothrombin time was prolonged the clotting time was prolonged to 46 minutes and the prothrombin consumption time was considerably decreased After the bleeding started the intravenous fat was discontinued The patient s coagulogram and bromsulphalein test returned to normal in about 3 weeks

Patient 10 received 17 units of fat over a period of 9 days After 4 units of fat the platelets fell to 58 000 However there was no evidence of clinical bleeding Two days later the platelets had returned to normal and intravenous

fat was resumed He received 17 units of fat over a period of 5 days and began to bleed from the lower gastrointestinal tract At the time the platelets were *normal but the one stage prothrombin* time was prolonged and the clotting time was 19 minutes Therapy was discontinued and no further evidence of bleeding was noted

DISCUSSION

After the infusion of 10 or more units of intravenous fat emulsion the BSP is usually increased This returns to normal after the infusions are discontinued In all instances of bleeding there were changes in the coagulogram *but these* changes were not consistent In some the clotting time was prolonged in others the prothrombin consumption time was diminished The 3 patients who experienced bleeding had prolonged one stage prothrombin times On the other hand other patients had prolonged prothrombin times prolonged clotting times and appreciable diminution of the prothrombin consumption time without any evidence of bleeding Continued infusions to these patients might have caused some bleeding

In most instances the changes in liver function and the coagulogram were reversible ones It would appear that the administration of 10 to 14 units of intravenous fat is safe Should it be necessary to continue the therapy it would be wise to follow liver function and the coagulogram If appreciable changes were found in the prothrombin clotting or prothrombin consumption times therapy should be discontinued for a few days until the coagulogram returned to normal and then further infusions given The fall in hematocrit is a very *consistent finding and when appreciable amounts of intravenous fat emulsion* are given hematocrits should be followed very closely

SUMMARY

Eleven patients who received from 12 to 42 units of the fat emulsion at the rate of 2 units daily were followed during the period with liver function tests and coagulograms at regular intervals All patients had a fall in hematocrit *In more than half the patients the plasma hemoglobin* was increased

All patients had an increase in bromsulphalein retention In most instances this returned to normal after the cessation of therapy Two patients became jaundiced clinically and half the patients had significant rises in serum bilirubin

Three patients experienced bleeding episodes after 42 32 and 17 units of intravenous fat emulsion Changes in the coagulogram reverted to normal in two of the patients after the cessation of therapy

Several of the patients had an increase in the one stage prothrombin time the Lee and White clotting time and a lowered prothrombin consumption time

It is suggested that 10 to 14 units of fat emulsion are safe Should an increased amount be necessary for appropriate therapy liver function tests and coagulograms should be carried out at regular intervals Any evidence of change in the coagulogram should warn of impending danger and therapy discontinued After the coagulogram returns to normal therapy might be reinstituted

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PERFORMANCE IN AN ACUTE COLD STRESS OF ANIMALS HAVING DIFFERING SATURATION OF BODY FATS*

Michael Hume William W L Glenn and Joseph J Barboriak

Any search for methods of extending the limits of cold tolerance invariably leads to a consideration of hibernation One of the known differences which distinguish hibernators concerns the saturation of the body fat Hibernators deposit a fat which is more unsaturated which remains liquid or semiliquid at the very low body temperature of the dormant state and the saturation of which decreases during the period before hibernation [1] It would appear to be an advantage to have a source of stored energy which is available for trans fer at low body temperature Non hibernators do not have the ability to de hydrogenate dietary fat to this degree and their body fat type is largely determined by diet Anderson and Mendel of this institution demonstrated that for the rat a non hibernator, the composition of body fat resembles quite closely that of ingested fat especially for highly unsaturated fats such as soy bean corn cottonseed and peanut oils (iodine numbers 132 121 108 and 102 respectively) whereas dietary fats of a lower iodine number such as lard butter, and coconut (63 36 and 8) were unsaturated (to 70, 55 and 35 respec tively) [2] One might conjecture that if the tolerance to cold of a non hibernator such as the rat might be improved by artificially altering the fat type so as to make it resemble more that of a natural hibernator, direct evidence would be available that tolerance of very low temperature is related at least in part to the type of body fat The present experiment explored this question by determining the resistance of the rat to an acute cold stress after this animal had been fed diets of widely differing fat type It was demonstrated to be a disadvantage to the animal to undergo this cold stress with highly saturated fat

METHOD

Male Sprague Dawley strain albino rats were divided into three groups at about 3 weeks of age These groups were fed a high carbohydrate control diet (fat content 10%) or a high fat diet (fat content 61 6%) consisting principally of either corn oil or coconut oil The diets which were similar in respect to calories vitamins minerals and proteins have been published in detail pre viously [3] The animals were housed in an air conditioned room in which the air temperature was kept at 70 to 72°F and relative humidity at 40 to 45% When the animals were about 8 weeks of age acute cold stress was carried out as described below The cold stress technique was modified from a method described by Andjus and Smith [4]

After 12 to 15 hours fasting the animal was placed in a wide mouth pickle jar within a deep-freeze the ambient temperature of which varied from −13 to −3°C during the period of cooling After 2 hours the animal was observed closely at intervals for loss of the righting reflex as the jar was rotated, and was removed at that time Light surgical anesthesia was present and permitted subsequent preparations Weight was recorded and the animal was strapped

* From ... versity School ... Victoria Fund

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MICHAEL HUME, WILLIAM W. L. GLENN, AND JOSEPH J. BARBORIAK

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* From the Department of Surgery and the Nutrition Laboratory, Yale University School of Medicine Supported by the James Hudson Brown Memorial Fund and the Victoria Fund for Cardiovascular Research

to a wire frame EKG needle electrodes inserted and a thermistor placed 5 to 8 cm into the rectum This placed the tip of the thermistor almost at the level of the diaphragm Baseline EKG was recorded and the frame immersed in a bath of ice and water so that the neck and head remained above water At 19°C artificial ventilation with 100% oxygen was begun using a mechanical respirator and a tight fitting nose mask When asystole occurred as shown on continuous monitoring of EKG the head was wrapped in wet gauze and the frame left in the ice bath for a variable length of time Ten minutes prior to rewarming an endotracheal tube was inserted A small neck incision to visualize the larynx facilitated transoral intubation The thermistor was re moved from the rectum and inserted into the esophagus as far as the middle of the mediastinum Precordial warming with tap water at 43°C was begun when the desired length of asystole had ended Whole body warming was begun when the swallowing reflex was established The neck incision was closed with a suture of catgut and when adequate respiration had returned the respirator was stopped The endotracheal tube was aspirated as frequently as necessary to keep it free of secretions and to estimate the amount of pul monary edema It was removed when body temperature approached normal and active movements had begun The animal was then dried with a bath towel and when the righting reflex returned was replaced in the constant temperature animal room

All animals were autopsied Survivors were sacrificed 1 week after the cold stress The thoracic and abdominal viscera were examined In 28 unselected animals samples were collected from the subcutaneous fat of the groin the omentum testicular and perinephric fat pads These were ground with a little clean quartz sand centrifuged and the supernatant oil withdrawn Iodine numbers in duplicate were performed by the Hanus method and the results averaged

RESULTS

1 **Weight** Average body weight in grams was as follows carbohydrate 285 corn oil 298 coconut oil 295 In those groups having ultimate survivors the breakdown by performance was as follows carbohydrate survivors 280 fail ures 287 corn oil survivors 282 failures 303 Survival did not correlate with either extreme of body weight

2 **Rate of cooling** The coconut fed group struggled less cooled more rapidly and reached asystole sooner and at a higher body temperature than the others This was not because of smaller body mass The average weight of the animals fed coconut oil was above the average of all the animals

3 **Temperature at asystole** Rectal temperature when heart action ceased was not of prognostic significance Some animals survived which reached asystole only after prolonged immersion during which their body temperature fell to quite low levels

4 **Electrocardiograph** Heart rate fell progressively with cooling until about 17 to 15°C Below this a regular idioventricular rhythm persisted until just before asystole when the rhythm became irregular once again The charac teristics of the EKG during cooling and warming did not vary with the body fat type

5 **Autopsy** Varying degrees of circulatory failure in the non survivors was obvious at autopsy Dry air passages and intense congestion of the viscera was characteristic of those in which no return of circulation was indicated by com

pletely abnormal EKG. Foam and fluid in the air passages and congestion of viscera were characteristic of those having temporary return of circulation or of those early successes that subsequently failed to survive.

6. Fat analysis. Iodine numbers of the depot fat of 28 animals are presented in the accompanying table.

Table 1. Average of Iodine Numbers

(FIGURES IN PARENTHESES INDICATE NUMBER OF ANIMALS)

DIET TYPE	CARBOHYDRATE	CORN OIL	COCONUT OIL
Survivors	80 (5)	119 (3)	—
Failures	73 (8)	120 ([illegible])	42 (8)

7. Performance. In a preliminary series of 90 animals the duration of the stress was varied to determine for each diet group a duration of asystole from which half of the animals would survive and half not survive. Thus the period between asystole and rewarming was varied in a stepwise fashion and success was arbitrarily set at the point of spontaneous adequate respirations and the ability to maintain an erect posture. This series demonstrated the following facts. First, that successes and failures were scattered at any duration of asystole selected with such overlapping among the diet groups that the mean of each group was not significantly distinct from the other groups. Second, it became apparent that in each diet group about half could be and half could not be resuscitated. Third, it appeared that among the corn oil fed group more of the early successes went on to ultimate survival than occurred in the other diet groups. Table 2 summarizes the performance of this preliminary series, but because ultimate survival was not a criterion of success selected at the beginning of the series, the record is not complete in this particular.

Table 2. Performance, Preliminary Series

DIET GROUP	CARBOHYDRATE	CORN OIL	COCONUT OIL
Number of animals in group	36	28	26
1. Failure of resuscitation	16	13	10
2. Early success	20	15	16
(a) Died subsequently	11	7	10
(b) Ultimate survival	0	6	1
(c) Record incomplete	9	2	5

A second series of 65 animals was prepared in order to study ultimate survival. Within each dietary group about one third of the animals were rewarmed after 50 minutes asystole, one third after 70 minutes and one-third after 90 minutes. Again, in this series resuscitation succeeded in only about half of the animals to the point at which spontaneous adequate respirations had been established and the righting reflex regained. However, ultimate survival

was achieved only among the corn oil fed and carbohydrate fed groups There were no survivors among the coconut oil fed group The performance of this series is summarized in Table 3

Table 3 Performance Second Series

DIET GROUP	CARBOHYDRATE	CORN OIL	COCONUT OIL
Number of animals in group	22	22	21
1 Failure of resuscitation	12	10	10
2 Early success	10	12	11
(a) Died subsequently	3	7	11
(b) Ultimate survival	7	5	0

DISCUSSION

Spontaneous adequate respirations after warming and the ability to stand steadily was considered early success In both series somewhat more than half of the animals without regard to diet achieved this early success The animals that could not be resuscitated exhibited abnormal EKG patterns characterized by arrhythmias and conduction defects If as Nardone[5] has suggested there is greater susceptibility to cold of specialized (conductive) than nonspecialized myocardial tissue these are the animals in which such a greater susceptibility of conducting tissue prevented resuscitation

Correlation of performance with diet could be made in the phase of recovery after initial resuscitation The two series cannot be combined in this phase because the criterion of success was modified in the second series on the basis of information gained in the first Nevertheless the incomplete results of the first series do not tend to contradict the results observed in the second Swan has described the phase after initial resuscitation as a period of relative myocardial insufficiency existing for several hours after rewarming characterized by rapid heart rate low cardiac output high peripheral resistance and normal oxygen consumption[6] This period then merges with recovery of normal cardiodynamics in those animals that recover and with ventricular failure in those that do not It was observed in the second series that all of the coconut fed animals that were resuscitated initially succumbed during this phase

The experiment was not designed to pinpoint the location of the defect which resulted in the poor performance of the animals fed on saturated fat What evidence is available of the structural and metabolic role of lipids in the living tissues does not furnish much direction to speculation about such a defect Autopsy of non survivors focused attention on the cardiovascular system In these there was evidence of myocardial failure Lipids are concerned in the structure of the cell membrane of all cells including the myocardium Finean[7] has demonstrated by x ray diffusion patterns (of myelin) alternating layers of lipid and protein in the cell membrane the fundamental repeating unit most probably consisting of two bimolecular leaflets of lipid and two protein layers The effect of cold on cell membrane physiology has been reviewed by Hoffman[8] Lipids have been demonstrated in the nucleus of the cell in the mitochondria microsomes and supernatant phase of the

cytoplasm[9] The function of all enzyme systems is somewhat temperature dependent This probably applies to intracellular metabolism Finally, the role of the unesterified fatty acid fraction of the plasma as a major metabolic link between depots and the liver and the periphery has received emphasis recently[10] It remains for further investigation to reveal what structural or metabolic function of the lipids evolved was responsible for the variation in performance of the animals in this experiment

The authors wish to express their great appreciation to Drs George R Cowgill and Willard A Krehl for their interest and suggestions and to Dr Colin White for statistical analysis

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THE METABOLISM OF ETHANOL IN MAN *

DAVID SELIGSON, H H STONE PAUL NEMIR JR.

A previous report[1] indicated that when alcohol was being metabolized by man blood flowing from the liver showed an increased concentration of lactate a decreased concentration of pyruvate a fall in glucose and a slight acidosis These findings plus those to be presented in this report are compatible with a framework for viewing the metabolism of alcohol in man and its chemical consequences These studies are from a program designed to compare the response of normal persons given alcohol with the response of alcoholics

* From the Departments of Biochemistry Medicine Surgery and Anesthesiology the Graduate Hospital and Graduate School of Medicine of the University of Pennsylvania Philadelphia Aided by a grant from the Commonwealth of Pennsylvania for the study of alcoholism and by a grant H 1289(c)4 N I H United States Public Health Service

METHOD

Nine male volunteers, ages 21 to 48, were studied. Under properly monitored conditions in an operating room catheters were placed in a femoral artery, antecubital vein and hepatic vein. Samples of blood were obtained for control values at minus 15 minutes and zero time. One liter of 5% alcohol in saline was administered in 20 minutes. Samples were obtained from each catheter at 30, 60 and 90 minutes, after the start of the alcohol. All samples were analyzed for lactate, pyruvate, β hydroxybutyrate, acetoacetate and total organic acids. The subjects responded to this dose of alcohol by becoming more loquacious but not drunk. Blood alcohol rose to approximately 80 mg % at 30 minutes and fell to approximately 55 mg % at 90 minutes.

RESULTS

Figure 1 shows the response of lactate, pyruvate, β hydroxybutyrate and acetoacetate in hepatic vein blood. The values shown are percentages of the *control values in order to avoid the variability of absolute values shown by* individuals. The mean control values were: lactate 6.4, pyruvate 0.43, β hydroxybutyrate 2.2 and acetoacetate 1.9 mg %. When lactate rises pyruvate falls and similarly when β hydroxybutyrate rises acetoacetate falls. Expressing these observations another way, the lactate/pyruvate ratio was 15 before alcohol and 70, 82 and 55 at 30, 60 and 90 minutes after alcohol respectively while the β hydroxybutyrate/acetoacetate ratio was 1.2 before alcohol and 2.3, 2.3 and 2.0 at 30, 60 and 90 minutes after alcohol.

Figure 2 shows a rise in organic acids in hepatic vein blood after alcohol.

DISCUSSION

Alcohol is oxidized by 2 steps of dehydrogenation (as shown in Figure 3) to produce acetic acid. In this process each molecule of alcohol yields 4 atoms of hydrogen. In these studies the alcohol administered intravenously was

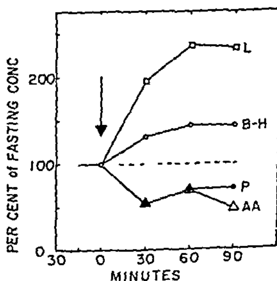


Fig 1 % change of lactate (–□–□–) β hydroxybutyrate (–○–○– B H) pyruvate (–●–●– P) and acetoacetate (–△–△– A) in hepatic vein blood after the administration of alcohol

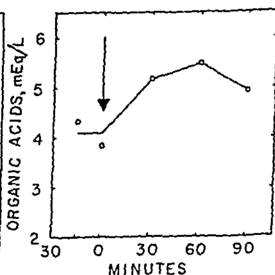


Fig 2 Change in organic acids in hepatic vein blood after the administration of alcohol

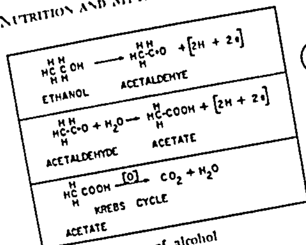


Fig 3 Oxidation of alcohol

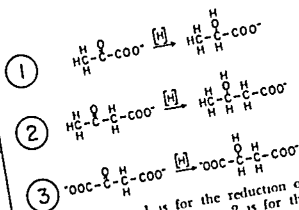


Fig 4 Equation 1 is for the reduction of pyruvate to lactate Equation 2 is for the reduction of acetoacetate to β hydroxybutyrate Equation 3 is for the reduction of oxaloacetate to malate

equivalent to three ounces of whisky The content of alcohol is approximately 1 mole and should yield on oxidation 1,000 mEq of hydrogen From general experience it is known that most of the alcohol and this amount of hydrogen is handled easily in a few hours The oxidizing agent *in vivo* is diphosphopyridine nucleotide (DPN) and the catalysts for the oxidation are the enzymes alcohol and aldehyde dehydrogenases In the oxidation DPN is reduced to $DPNH_2$, in other words 2 molecules of hydrogen pass from the alcohol to DPN The liver contains approximately 3 mM of DPN to accomplish the oxidation of the 1,000 mM of alcohol or participate in the transfer of 1,000 mEq of hydrogen The $DPNH_2$ which is formed passes its hydrogen on to various acceptors and in the process is oxidized to DPN which can again participate in the oxidation of alcohol Normally, the hydrogen of $DPNH_2$ is passed on to the respiratory chain of enzymes and coenzymes and finally to oxygen to become water It would appear from the data that when large amounts of hydrogen are being rapidly transported other acceptors than the respiratory chain of enzymes and coenzymes accept hydrogen In this case pyruvate accepts hydrogen (in the presence of lactic dehydrogenase) and becomes lactate while acetoacetate becomes β hydroxybutyrate (Figure 4) These hydroxy acids are probably relatively inert and contribute to the rising pool of organic acids following the metabolism of alcohol as shown in Figure 2

Probably other keto acids participate as temporary hydrogen acceptors under similar circumstances For example, it is possible that some oxaloacetic acid is reduced to malic acid (Figure 4) during the metabolism of alcohol If these compounds, such as oxaloacetate or pyruvate, are essential for the functioning of metabolic cycles such as the citric acid cycle, reduction by an excess of hydrogen to the hydroxy acids and removal from the cycle could lead to a lower rate of activity in the citric acid cycle during the acute phase of alcohol metabolism Such may be the case since we have observed a diminution of adenosine triphosphate in the livers of rabbits, rats and guinea pigs after the administration of alcohol

Healthy persons appear to handle easily the large amount of hydrogen produced in the oxidation of alcohol presumably because they can provide temporary acceptors for hydrogen For example, liver containing adequate stores

of glycogen can make pyruvate in large amounts which temporarily is converted to lactate If glycogen were converted effectively to pyruvate and pyruvate to lactate 1 gram of glycogen could accept 25 mM of hydrogen It has been observed that glycogen diminishes during the metabolism of alcohol In depleted persons therefore it is conceivable that alcohol could have a more deleterious effect than in healthy persons

SUMMARY

In man the oxidation of moderate amounts of alcohol leads to an increase in the lactate to pyruvate ratio and β hydroxybutyrate to acetoacetate ratio in hepatic vein blood Total organic acids also rise after alcohol It is suggested that a consequence of the metabolism of alcohol is a temporary inhibiting effect on other metabolic pools

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THE EFFECT OF AUTONOMIC NERVE SECTION UPON THE INTESTINAL ABSORPTION OF GLUCOSE *

ROGER MESMER HARRY W HALE JR MANUEL SCHREIBER

We alter autonomic activity in our daily medical practices hoping to cure a variety of ills in which we implicate abnormal autonomic function By these regimens medical and surgical we accomplish relief or cure confirming our belief that irregularities of autonomic function cause disease Yet in some organs particularly the small intestine our knowledge of autonomic physiology is far from complete Many variables make conclusions hard to obtain The present study of glucose absorption and venous drainage of absorbing jejunum offers a little more light for this dark area

Previous studies of glucose absorption in vagotomized and in sympathectomized animals arrived at variant conclusions In 1933 Horne McDougall and Magee [1] noted in the ear vein of the rabbit a 63% increase in blood sugar after vagotomy and a 92% increase after sympathectomy when 10% glucose was injected into the ligated small intestine Niccolini [2] a year earlier had reported decreased blood sugar levels following vagotomy

METHOD

Healthy mongrel dogs were fasted 24 hours They were anesthetized with intravenous pentothal and the abdomens were opened in the midline They

* From the Edward J Meyer Memorial Hospital Department of Surgery and University of Buffalo

were heparinized. Portal venous blood samples were drawn. The superior mesenteric vein branch immediately distal to the ligament of Treitz was catheterized with polyethylene tubing, and the segment of jejunum drained by this vein was isolated with two Kocher clamps Ten milliliters of 10% glucose was injected through a twenty gauge needle into the gut lumen and the loop was returned into the abdomen. After the 30 minute absorption period the loop of gut was excised; its contents were collected and it was weighed. Ten per cent dilutions were immediately made in zinc sulfate and barium hydroxide of both the succus and plasma at zero and 30 minutes. Vagotomies were performed by removing two inch segments of both thoracic vagi a week prior to absorption study. Sympathectomies consisted of removal of the thoracic chain from T6 to L1 in two stages 1 and 2 weeks prior to study. The criterion of absorption was the number of mg. of glucose found in the blood drained from the isolated loop of gut during the 30 minute test period.

RESULTS

After vagotomy the amount of glucose absorbed recorded as mg. of glucose per gm of absorbing gut, was diminished from 3 5 in the control series of 12 dogs to 1.1 in 9 vagotomized dogs In the 9 sympathectomized dogs, the mg. per gm rose to 12.3 as shown in Figure 1. Figure 2 shows increased venous drainage after sympathectomy from 2 5 to 5 2 ml. gm. of absorbing gut. Vagotomy did not significantly alter the venous drainage volume. Figure 3 shows that neither vagotomy nor sympathectomy significantly alters the volume of succus in the absorbing loop of intestine.

PREPARATION	CC GLUCOSE/GRAM MEAN	RANGE
Control (12)	3.5	2.2 to 4.5
Vagotomy (9)	1 1	0 4 to 3 3
Sympathectomy (9)	13 3	5 1 to 14 0

Glucose absorbed as mg per gm of gut in 30 minutes by a loop of jejunum Ten centimeters 5% glucose in loop

Fig 1

PREPARATION	CC BLOOD/GRAM MEAN	RANGE
Control	2 7	1 6 to 3 0
Vagotomy	3 2	2 1 to 5 9
Sympathectomy	5 2	2 5 to 8 0

Venous drainage of jejunal loop as cc per gm of gut in 30 minutes Ten centimeters 5% glucose in loop

Fig 2

PREPARATION	CC SUCCUS/GRAM MEAN	RANGE
Control	57	4 to 6
Vagotomy	62	38 to 9
Sympathectomy	55	55 to 83

Intestinal content as cc per gm of gut in 30 minutes Ten centimeters 5% glucose in loop

Fig 3

DISCUSSION

One would expect that section of the vagus which increases vascularity, motility, and secretion would decrease absorption as this experiment shows

it has done Wright[3] has shown that subcutaneous injection of eserine produces copious secretions from the whole small intestine due to hypermotility Section of the sympathetic also induces hypermotility Intravenous epinephrine causes cessation of intestinal movements and constriction of intestinal blood vessels with blanching of mucosa Yet this effect of hypermotility is not the cause of the present findings because in the opened abdomen, these clamped loops of gut displayed no motility Kokas and Ludany[4] found that vagal stimulation caused increased movement of villi, the villi contract and their capillaries are dilated Splanchnic nerve stimulation causes the villi to contract and their capillaries to be constricted It therefore might be assumed that the increased absorption after sympathectomy is on the basis of capillary dilatation and increased muscosal activity

However, it was following vagotomy that venous drainage was significantly increased Sympathectomy caused only slightly increased venous drainage Wolf and Wolff's[5] fistulous subject, Tom demonstrated mucosal engorgement upon prolonged emotion Kokas and Ludany demonstrated increased mucosal activity with widened capillaries upon stimulation of the vagus The finding of increased venous drainage postvagotomy is therefore surprising in the light of the literature It rules out increased vascularity as the cause of increased absorption in these sympathectomized dogs

Even more surprising is the lack of difference in intestinal luminal volume in loops of absorbing jejunum after autonomic nerve section The picture of denervated gut reported in the literature is not thus Wright found that a copious secretion from the duodenum could be produced by sectioning the thoracic greater splanchnic nerves, by cutting all preganglionic fibers, increased secretion occurred in the entire small intestine This paralytic secretion is ascribed to vasodilatation and increased motility, the latter of which does not occur in the present study

The difficulties in obtaining accurate knowledge of the workings of the autonomic nervous system upon the small intestine are well seen in the present study Opening the abdomen itself stimulates nerve endings in muscle and mucosa and depresses peristalses Factors in external environment have to be minimized by keeping experimental conditions identical in each animal Absorption capacity diminishes as we descend the gut, therefore segments studied have to
itself may alter
phenobarbital does not alter the effect of sympathetic stimulation but pento thal does have a vagolytic effect as shown by quickening of pulse The rather small differences between the controls and the vagotomized dogs in the present study indicate that some vagal activity has been depressed in the controls Much of the confusion in studying autonomic activity in the small intestine is caused by the mixed nature of these nerves They contain sensory as well as both pre and postganglionic fibers and therefore have varying effects often depending on strength of stimulation

These studies in the dog indicate that interruption of vagal stimulation of intestinal secretion and motility will diminish the absorption of glucose and that sympathectomy produces increased absorption This may help to explain the frequent weight loss seen in postgastrectomy patients The therapeutic vagotomy or the unavoidable vagus section in high gastric resection may considerably decrease intestinal absorption of glucose and other foodstuffs

SUMMARY

1 Vagotomy is followed by decreased glucose absorption Sympathectomy is followed by increased absorption

2 Vagotomy is followed by increased venous drainage

3 Neither vagotomy nor sympathectomy produce a change in succus volume

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CHANGES IN HUMAN CITRATED PLASMA PRODUCED BY 60°C HEAT EXPOSURE FOR 10 HOURS AND THE PROTECTIVE ACTION OF FORMALDEHYDE AND DEXTROSE*

ABDUL AL SHAMMA WILLIAM W PFAFF EDWARD A STEMMER AND J GARROTT ALLEN

Homologous serum jaundice remains a serious problem of transfusion therapy Many attempts have been made to inactivate the causative agent in pooled human plasma Most methods that claim promise have not been subjected to clinical testing Despite the proven safety[1, 2] of liquid pooled plasma after six months storage at 32°C many believe this procedure impractical for large scale use First the temperature range of 32°C is highly suitable for bacterial proliferation if contamination has occurred Therefore, all plasma must be bacteriologically sterile prior to storage at 32°C While this can be easily accomplished by blood banks most of the pharmaceutical industry has had no experience with asepsis in the manufacture of sterile biologicals and therefore are reluctant to consider this method of plasma production within their capacities to achieve Second the six month storage precludes the rapid accumulation of a supply of plasma in a time of emergency

Gellis *et al* have shown that albumin which had been contaminated with icterogenic plasma lost infectivity for human volunteers when heated at 60°C for 10 hours[3] Certainly clinical experience has amplified his observations and suggests that this amount of heat may also be adequate to sterilize plasma However under these circumstances most of the nonalbumin plasma proteins undergo denaturation including frank coagulation The addition of suitable agents will minimize the alteration of plasma proteins to heat but the

* From the William H Danforth Laboratory for Research in Surgery Department of Surgery University of Chicago Supported in part by a grant from the Surgeon General s Office of the U.S Army Contract No DA-49 007 MD 93

question remains the full significance of these changes is unknown so far as the transfusion value of such plasmas is concerned

In this study, plasma has been prepared by a variety of bactericidal methods which are known or have been shown herein, to produce severe alterations in the electrophoretic patterns of plasma as well as in other physicochemical measurements Thus a number of additives have been used to minimize the changes observed One of these products has been tested in dogs, infusing about 100 ml of plasma daily for one month with no difficulty Among the additives tested are dextrose, caprylate, acetyl tryptophanate, acetaldehyde ethyl acetoacetate, bromoacetate, chloroacetate and formaldehyde Of all these agents, chloroacetate and formaldehyde in combination with dextrose in a mild alkaline solution were found to be most promising Evaluation of halogenated acetates is still in progress

The present paper deals with the *in vitro* evaluation of formaldehyde in its ability to stabilize plasma proteins heated at 60°C for 10 hours, thus combining the strenuous effects of heat denaturation with that of chemical fixation

METHOD

Freshly pooled citrated human plasma was employed throughout in this study Formaldehyde in suitable quantities was then added to produce the desired final concentrations 1 1 000, 1 2,000 and 1 5,000

Formaldehyde concentration in plasma was measured by the modified rosaniline hydrochloride method after protein precipitation in acid medium [4] This enabled an estimate of the free as well as the combined or fixed formaldehyde Optical density at 610 mμ of the plasma products tested was measured by a Coleman, Jr spectrophotometer. Viscosity was read by Hellige technique, and paper electrophoresis was obtained by a Durrham Spinco apparatus The pH of the products was measured by a Cambridge pH meter, Research Model

RESULTS

Freshly pooled citrated human plasma was divided into two lots One lot was used as a control adding enough distilled water to make a 90% plasma solution To the second lot, the following reagents were added enough NaOH, formaldehyde and dextrose to increase the pH to 7 80 and to make a final concentration of 1 5,000 formaldehyde and 5% dextrose in plasma The final solution contained 90% plasma Aliquots of both these lots were heated at 60°C and hourly measurements of pH, optical density, viscosity and electrophoresis for 10 hours were recorded

The results of these procedures are shown in Figures 1, 2, 3 and 4 Precipitation of fibrinogen and of many globulins occurred within less than one hour when normal plasma was heated at 60°C This change increased thereafter, although the major part of this alteration had occurred during the first hour An initial and progressive rise in viscosity was also recorded (Fig 1) The electrophoretic pattern revealed a rapid decrease in the albumin globulin ratio, coincidental with the progressive appearance of an inhomogenous peak within the range usually considered as globulins This abnormal fraction or group of fractions lost mobility and gradually approached the starting point (Fig 2)

Similarly, in the plasma containing 5% dextrose and then treated with formaldehyde, most of the changes that were present at the end of 10 hours had

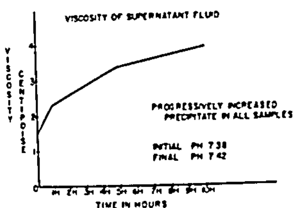


Fig 1 Pooled citrated human plasma heated at 60°C. for 10 hours showing changes in viscosity

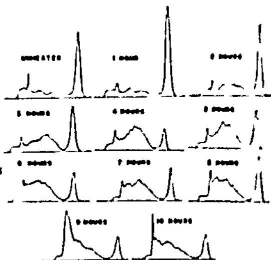


Fig 2 Hourly determination of electrophoretic pattern of plasma proteins after heating of human plasma at 60°C for 10 hours

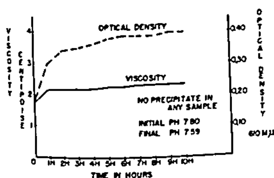


Fig 3 Pooled citrated human plasma treated with 1 5 000 formaldehyde and 5% dextrose and then heated at 60°C for 10 hours Hourly measurements of viscosity and optical density are shown

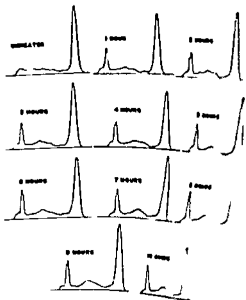


Fig 4 Pooled citrated hu[illegible] ing hourly changes in the [illegible] patterns of pooled citrate[illegible] treated with 1 5 000 forma[illegible] dextrose during the co[illegible] 60°C for 10 hours

already occurred by the end of one hour However, precipi[illegible] cur and the solution remained clear with only slight increa[illegible] sity and a moderate increase in viscosity (Fig 3) Electroph[illegible] vealed a well preserved albumin peak there was a loss of [illegible]

various globulin fractions but the mobility of the globulins, unlike untreated plasma after heating, was maintained (Fig 4) The residual free formaldehyde concentration in the 10 hour sample was 1 25,000, suggesting that about 80% of the initial 1 5,000 concentration of formaldehyde was protein bound ' The initial pH was 7 80 and the 10 hour sample pH was 7 59

DISCUSSION

It is apparent that plasma containing 1 5,000 formaldehyde and 5% dextrose in a mildly alkaline medium withstands heating at 60°C for 10 hours with much less denaturation than when plasma is heated without these agents

The reaction between formaldehyde and protein is biphasic First, formaldehyde is hydrated, forming a diol which then reacts with free amino groups to form an amino methanol compound [5] This reaction is fairly stable, and when a basic aromatic amino group is involved, the combination is said to be irreversible, resisting hydrolysis even in strong acids [4]

When the plasma proteins are combined with formaldehyde, their physical and chemical behaviors are profoundly altered The thermal stability of protein is increased, however, when formaldehyde is added, due to blocking of its reactive amino groups, and thereby preventing or interfering to some extent with inter or intramolecular binding and the consequent denaturation For the same reason the electrophoretic pattern of proteins is changed due to the alteration in the electric charge such treatment induces Thus part of the electrophoretic change in this plasma preparation is due to formaldehyde protein reaction and part is due to heat The fact that most of the alterations observed at the end of 10 hours of heating were noted at the end of the first hour suggests that most of the formaldehyde protein reaction occurred during the first hour, thus affording protection against subsequent heating Sodium hydroxide was used to increase the pH of this preparation to 7 80 in order to accelerate the formaldehyde protein reaction [5]

Glucose exerts a heat stabilizing effect on plasma proteins and is routinely used by some to prevent precipitation of fibrinogen in plasma [6] The mechanism which enables glucose to protect plasma against heat denaturation is not clearly understood, there is some evidence to suggest that it may act through its oxidation products Glucose also enhances the action of formaldehyde in preventing thermal coagulation of plasma proteins

Formaldehyde at 1 5,000 concentration is virucidal for many agents It is one of the most effective virucidal agents available, but we have no knowledge of such action with reference to the virus of homologous serum jaundice [7] In addition, at this concentration formaldehyde is effectively bactericidal for non sporing bacteria, thus it offers a safeguard in the handling of plasma prior to heating As shown by Pfaff *et al*, this treatment of plasma does not interfere with its nutritional value, increase reaction rates or produce detectable toxic effects in dogs transfused daily with canine plasma similarly treated

SUMMARY

1 The addition of 1 5,000 formaldehyde and 5% dextrose in pooled citrated human plasma enables the plasma to withstand heating at 60°C for 10 hours with minimal alterations Therefore, a bacterially sterile plasma preparation is provided, despite willful bacterial contamination

2 The changes in electrophoretic patterns, optical density and viscosity do

not appear to have affected the tolerance of dogs for canine plasma subjected to heat and formaldehyde Although it is possible that these changes may make homologous plasma antigenic, this is not yet demonstrated

3 A variety of other treatments for plasma were similarly examined and will be reported elsewhere Some of these appear more useful than the combinations presented in this report

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THE TREATMENT OF POOLED HUMAN PLASMA WITH FORMALDEHYDE AT 60°C FOR TEN HOURS*

Biologic Studies

William W Peale Edward A Stemmer Abdul Al Shamma Donald Dawson and J Garrott Allen

In this study are the results of tolerance and metabolic studies of dogs for intravenous infusion of homologous formol treated plasma given daily for 28 days when this was the major source of protein

METHOD

The mongrel dogs used in this experiment weighed between 8 0 and 11 6 kg the design of the experiment called for 3 groups each group consisted of 2 animals 1 male and 1 female All animals had been maintained under observation for at least 2 months prior to participation in this study and were known to be free from infection including alimentary parasitic disease Each animal received by subcutaneous injection 0 5 ml of canine anti-distemper serum per kg of body weight each week throughout the control and test periods

Basal Diet All 6 animals were maintained on subminimal protein through the 2 week control period [illegible]
lowed One hundred calo
control and test periods

* From the William H Danforth Laboratory for Research in Surgery Department of Surgery University of Chicago Supported in part by a grant from the Surgeon General's Office of the U S Army Contract No DA 49 007 MD 93

We are indebted to Dr Eleanor M Humphreys Professor in the Department of Surgical Pathology University of Chicago for her extensive help in the evaluation of the pathological slides used in connection with this experiment

various globulin fractions but the mobility of the globulins unlike untreated plasma after heating was maintained (Fig 4) The residual free formaldehyde concentration in the 10 hour sample was 1 25 000 suggesting that about 80% of the initial 1 5 000 concentration of formaldehyde was protein bound The initial pH was 7 80 and the 10 hour sample pH was 7 59

DISCUSSION

It is apparent that plasma containing 1 5 000 formaldehyde and 5% dextrose in a mildly alkaline medium withstands heating at 60°C for 10 hours with much less denaturation than when plasma is heated without these agents

The reaction between formaldehyde and protein is biphasic First formaldehyde is hydrated forming a diol which then reacts with free amino groups to form an amino methanol compound [5] This reaction is fairly stable and when a basic aromatic amino group is involved the combination is said to be irreversible resisting hydrolysis even in strong acids [4]

When the plasma proteins are combined with formaldehyde their physical and chemical behaviors are profoundly altered The thermal stability of protein is increased however when formaldehyde is added due to blocking of its reactive amino groups and thereby preventing or interfering to some extent with inter or intramolecular binding and the consequent denaturation For the same reason the electrophoretic pattern of proteins is changed due to the alteration in the electric charge such treatment induces Thus part of the electrophoretic change in this plasma preparation is due to formaldehyde-protein reaction and part is due to heat The fact that most of the alterations observed at the end of 10 hours of heating were noted at the end of the first hour suggests that most of the formaldehyde protein reaction occurred during the first hour thus affording protection against subsequent heating Sodium hydroxide was used to increase the pH of this preparation to 7 80 in order to accelerate the formaldehyde protein reaction [5]

Glucose exerts a heat stabilizing effect on plasma proteins and is routinely used by some to prevent precipitation of fibrinogen in plasma [6] The mechanism which enables glucose to protect plasma against heat denaturation is not clearly understood there is some evidence to suggest that it may act through its oxidation products Glucose also enhances the action of formaldehyde in preventing thermal coagulation of plasma proteins

Formaldehyde at 1 5 000 concentration is virucidal for many agents It is one of the most effective virucidal agents available but we have no knowledge of such action with reference to the virus of homologous serum jaundice [7] In addition at this concentration formaldehyde is effectively bactericidal for non sporing bacteria thus it offers a safeguard in the handling of plasma prior to heating As shown by Pfaff *et al* this treatment of plasma does not interfere with its nutritional value increase reaction rates or produce detectable toxic effects in dogs transfused daily with canine plasma similarly treated

SUMMARY

1 The addition of 1 5 000 formaldehyde and 5% dextrose in pooled citrated human plasma enables the plasma to withstand heating at 60°C for 10 hours with minimal alterations Therefore a bacterially sterile plasma preparation is provided despite willful bacterial contamination

2 The changes in electrophoretic patterns optical density and viscosity do

not appear to have affected the tolerance of dogs for canine plasma subjected to heat and formaldehyde Although it is possible that these changes may make homologous plasma antigenic, this is not yet demonstrated

3 A variety of other treatments for plasma were similarly examined and will be reported elsewhere Some of these appear more useful than the combinations presented in this report

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THE TREATMENT OF POOLED HUMAN PLASMA WITH FORMALDEHYDE AT 60°C FOR TEN HOURS *

Biologic Studies

WILLIAM W PEATT EDWARD A STEMMER ABDUL AL SHAMMA DONALD DAWSON AND J GARROTT ALLEN

In this study are the results of tolerance and metabolic studies of dogs for intravenous infusion of homologous formol treated plasma given daily for 28 days when this was the major source of protein

METHOD

The mongrel dogs used in this experiment weighed between 8 0 and 11 6 kg the design of the experiment called for 3 groups each group consisted of 2 animals 1 male and 1 female All animals had been maintained under observation for at least 2 months prior to participation in this study and were known to be free from infection including alimentary parasitic disease Each animal received by subcutaneous injection 0 5 ml of canine anti-distemper serum per kg of body weight each week throughout the control and test periods

Basal Diet All 6 animals were maintained on subminimal protein through the 2 week control period and the 1 week test period which immediately followed One hundred calories per kg of body weight were allowed in both the control and test periods This basal diet was modified during the test only in

* From the William H Danforth Laboratory for Research in Surgery Department of Surgery University of Chicago Supported in part by a grant from the Surgeon General s Office of the U S Army Contract No DA 49 007 MD 93

We are indebted to Dr Eleanor M Humphreys Professor in the Department of Surgical Pathology University of Chicago for her extensive help in the evaluation of the pathological slides used in connection with this experiment

relation to its protein content, source and route of administration Fortunately, all dogs consumed their diet completely each day through the entire study. The composition of basal diet fed all animals throughout the entire study was as follows.

Table 1

		NO OF CALORIES	% OF TOTAL CALORIES
Sucrose	2720 gm	13,056	73 1
Dextrin	280 "	No calories	—
Agar	40 "	No calories	—
Wesson's Salt	160 "	768	4 3
Choline	12 "	57	09
Paba	0 6 "	3	01
Lard	600 "	2 880	17 0
Mazola	200 "	960	5 5
	Total Cal	17,714	100 0%

(One cake of 4,000 gm Mix was figured as 480 cal /100 gm)

Test Periods and Results. *Group A* (37–2 and 37–3): These 2 animals received only the basal diet containing 0 23 gm per kg of oral protein † This was given throughout the entire 6 week period to test the animal's response to this quantity of protein in conjunction with the basal diet The total calories allowed were 100 per kg of body weight The results are shown as follows and also include data from Groups B and C.

Table 2 Twenty-eight day Experimental Period

	DAILY CALORIES	TOTAL DAILY PROTEIN	WEIGHT CHANGE IN KG DURING 28 DAYS INITIAL	FINAL		WEIGHT CHANGE ±
Group A						
1 #37 2	850 cal	1 95 gm < oral	8.5	8.5	=	0 0
2 #37 3	860 cal	1 95 gm < oral	8.5	8 8	=	+0.3
Group B						
1 #37 4	810 cal	46 gm < oral	8 3	7 9	=	—0 4
2 #37 7	780 cal	44 gm < oral	7 8	7 4	=	—0 4
Group C						
1 #37 5	1150 cal	6 46 gm < oral 644; I V plasma 5 81	11 3	11 6	=	+0 3
2 #37 8	1010 cal	5 79 gm < oral 57; I V plasma 5 22	10 4	10 1	=	—0 3

Group B (37–4 and 37–7) They received the same basal diet as Group A, except that their oral protein was reduced after the first 2 weeks from 0 23 gm to 0 05 gm per kg of body weight for the remaining 4 weeks

Group C (37–5 and 37–8) They received the basal diet with 0 23 gm of protein by mouth during the first 2 weeks Thereafter the oral protein was

† Commercial house meat dog food (Rival Dog Food)

reduced to 0 057 gm per kg of body weight for the next 4 weeks However, beginning with the test period (after the first 2 weeks) these 2 animals received homologous citrated plasma to which was later added formaldehyde dextrose and sodium hydroxide (2 537 N) The final product was of the following composition

Table 3 Per Cent Composition of Formolized Citrated Canine Plasma

Plasma	80 0%
4% Citrate Sol	10 0%
2% Sol of Formaldehyde	0 9%
50% Dextrose	8 9%
2.537 N NaOH	0.2%
	100 0%

The final product contained 4 5 gm per cent of protein It was administered daily by vein in volumes sufficient to supply 0 5 gm of protein per kg of body weight (110 to 120 ml of plasma daily) Thus these 2 animals received 0 057 gm per kg orally and 0 50 gm per kg by vein each day for 28 days a total daily protein intake of 0 557 gm per kg This came to 6 4 gm (1 02 gm of nitrogen) of total protein per day for #37–5 and 5 9 gm (0 94 gm of nitrogen) daily for #37–8 These values were obviously greater than the total protein given the dogs in either Group A or B (see Table 2)

Nitrogen balance data representative for 1 animal in each of the three groups are shown in Figure 1 As might be expected with the extremely low protein diet all dogs were in negative nitrogen balance Noteworthy however is the fact that the 2 dogs on formolized plasma showed less of a negative balance than the animals in either of the other two groups (A & B) Dog #315 (receiving the plasma) was actually in positive nitrogen balance

Figures 2 and 3 show a composite of chemistries obtained on #37–2 and #37 5 of Groups A and D respectively

There was a decrease in the concentration of total plasma proteins including serum albumin in all 6 animals comprising the three groups with the exception of #37–5 which received formolized plasma The second animal receiving formolized plasma showed a minor decline in plasma protein concentration but this reduction was less than occurred in any of the animals comprising either Groups A or B

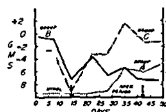


Fig 1 Comparison of nitrogen balance of low oral protein intake versus parenteral administration of formol treated plasma

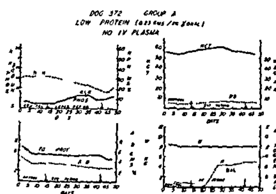


Fig 2 Composite results of chemistries obtained from low oral protein intake

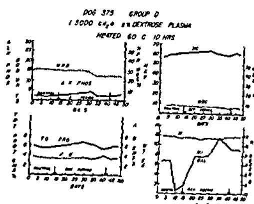


Fig 3 Composite results of chemistries obtained from parenteral administration of formol treated plasma

Alkaline phosphatase was elevated in all animals by the time the study period terminated However, the least change occurred in the animals receiving formolized plasma Serum bilirubins remained normal for all animals throughout the study

Electrophoretic patterns of the serum of dogs receiving formolized plasma were determined every third day throughout the control and test period Formaldehyde produces characteristic changes in the electrophoretic patterns as described by Al Shamma *et al* When this plasma is administered these changes are detected in the recipient animal within 24 hours after transfusion As the period of transfusion proceeds from day to day, a point is reached at which no further increase in these abnormal patterns appears in the serum of the recipient animal This suggests an equilibrium is reached wherein the transfused plasma is refabricated at a rate commensurate with the transfusions given

At the end of 28 days, all animals were subjected to biopsy of the liver, kidney, adrenals, and bone marrow These tissues were sectioned and stained with hematoxylin eosin and with Sudan III No abnormalities were detected in any of the three groups Failure to find at least some evidence of hepatic depression of protein was accounted for on the basis of sustained caloric intake and that the duration of study was not sufficiently great for protein depletion to become a serious problem

DISCUSSION

The purpose of this study was to examine the possibility that formolized plasma might be useful as a means to avoid bacterial growth should contamination occur in the preparation of liquid plasma Formaldehyde is also known to be virucidal On the other hand, formaldehyde is best known for its capacity to fix proteins and thereby to create profound denaturation It is therefore surprising that the animals given 100 to 110 ml of formolized plasma per day tolerated this product without the slightest clinical or laboratory evidence of unfavorable response in fact the data presented suggest the transfused plasma was readily available for nutritive purposes The possibility remains however, that the alteration in plasma proteins produced by formaldehyde may sensitize the animal and that upon administration of the same product at a later date some form of sensitivity may be manifest

THE SITE OF AMMONIA PRODUCTION AND ABSORPTION IN ECK FISTULA DOGS *

PAUL F. GRYSKA AND ERNEST M. BARSAMIAN

It is a common clinical observation that hyperammonemia and "hepatic coma" follow upper gastrointestinal hemorrhage in cirrhotic patients McDermott *et al.*[1] were able to produce a recognizable comatose state and eventual death by feeding whole blood to Eck fistula dogs in whom there was a concomitant blood ammonia elevation.

Theoretically, ammonia is produced when ingested protein is acted upon by *amino acid oxidase or urease. These enzymes are produced* by gastrointestinal bacteria.[2]

The purpose of this study is to define the site or sites in the gastrointestinal tract that are significant in the production and absorption of ammonia.

METHOD

An Eck fistula was created in 13 adult mongrel dogs by performing a side-to-side portacaval shunt with ligation of the portal vein on the hepatic side of the shunt. All dogs were allowed to convalesce for at least 30 days Four groups of at least three studies each were then carried out:

Group 1—250 cc. of out-dated human whole blood was given by tube into the stomach of unanesthetized dogs.

Group 2—250 cc. of whole blood was similarly inserted into the colon by enema.

Group 3—250 cc. of 5% urea was given into the stomach.

Group 4—250 cc. of 5% urea was given into the colon by enema.

All the *animals were starved for 24 hours prior to each study*. Blood ammonia was determined by the Conway microdiffusion method on heparinized peripheral venous blood samples These were drawn immediately prior to feeding and at intervals of 15 minutes, 30 minutes, 1, 2, 3 and 4 hours after administration of blood or urea

RESULTS

Table 1 is a tabulation of the changes in blood ammonia after intragastric or intracolonic feeding of blood or urea Whole blood given into the stomach produces a distinct rise of blood ammonia in 2 to 3 hours after feeding. However, when given into the colon by enema, there is less significant blood ammonia elevation during the period of the study (Fig 1) These observations suggest (1) that production and absorption of ammonia takes place in the more distal gut and (2) that blood must be digested before ammonia production can take place

Since ammonia production is felt to be a result of enzymatic action of intestinal bacteria on protein breakdown products, a similar group of observations were carried out with urea placed in the upper and lower gastrointestinal

* From the Department of Surgery, Harvard Medical School, Fifth Surgical Service, and Sears Surgical Laboratory, Boston City Hospital, aided in part by a grant from the Berkshire County Chapter of the Massachusetts Heart Association and by a research grant, H 3345, from the National Heart Institute, Public Health Service

Table 1 Tabulation of Blood Ammonia Levels (μg %) in Dogs With Eck Fistula After Administration of 250 cc Blood or Urea in Stomach or Colon

	Group 1 Blood in Stomach				Group 2 Blood in Colon			Group 3 Urea in Stomach			Group 4 Urea in Colon		
	Dog 3	4	1	Dog 5	6	2	13	Dog 7	11	12	Dog 8	9	12
Control (Pre administration)	465	393	626	364	283	554	609	571	368	499	453	413	352
15 min	465	388	599	419	275	582	817	571	335	476	443	486	396
30 min	486	404	413	352	275	618	810	590	332	539	510	588	449
1 hr	465	441	699	368	267	550	813	546	344	543	659	644	732
2 hrs	546	421	717	364	267	589	743	549	396	459	797	680	1154
3 hrs	638	465	599	368	368	547	669	626	498	366	—	571	1433
4 hrs	516	468	—	—	340	440	854	588	323	489	—	514	1070

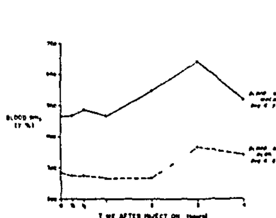


Fig 1 Typical effect on blood ammonia of 250 cc. of whole blood in proximal and distal gastrointestinal tract in dogs with Eck fistulae

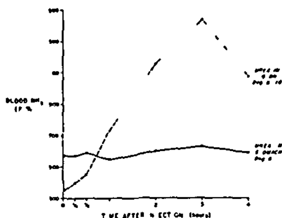


Fig 2 Typical effect on blood ammonia of 250 cc of 5% urea in proximal and distal gastrointestinal tract in dogs with Eck fistulae

tract (Fig 2) After intragastric administration of urea, there was a transitory late elevation of blood ammonia probably because some of the highly diffusible urea was absorbed in the small bowel and only a relatively small amount reached the lower gut When urea was given per rectum, the blood ammonia level began to rise within fifteen minutes and maintained markedly high levels This suggests that ammonia is produced and absorbed in the colon

In order to more conclusively demonstrate the colon as the site of ammonia production and absorption, one additional experiment was carried out (Fig 3) An intragastric feeding of 250 cc of whole blood was given to an Eck fistula dog with a previously created functioning ileostomy There was no appreciable rise in the blood ammonia during this period The ileostomy fluid consisting of blood and ileal fluid was collected and 250 cc of this was given into the colon of another Eck fistula dog The blood ammonia level of this dog rose to extremely high levels almost immediately The dog lapsed into coma, had convulsions and died with pronounced hyperammonemia

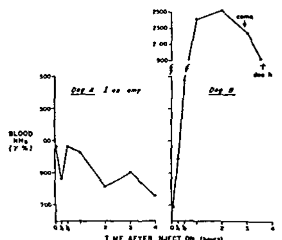


Fig 3 *Dog A* Effect on blood ammonia of 250 cc. whole blood placed in stomach of a dog with an Eck fistula and ileostomy
Dog B Effect on blood ammonia of 250 cc of ileostomy drainage including blood from Dog A placed in colon of Dog B with Eck fistula and intact gastrointestinal tract

CONCLUSIONS

These observations would appear to confirm the experiments of Silen *et al*[3] in which higher levels of blood ammonia were obtained from the colonic venous drainage than elsewhere after oral protein ingestion in dogs The rationale of the use of intestinal catharsis antibiotics and enemata to prevent dangerous ammonia levels in cases of bleeding esophageal varices is further substantiated by these observations

SUMMARY

In dogs with an Eck fistula

1 Blood ammonia levels are elevated when blood originates in the upper gastrointestinal tract

2 Protein or whole blood must be predigested before ammonia production can occur

3 This production and absorption occurs in the large bowel

4 Colonic bleeding alone will probably not produce an elevated blood ammonia level of significance

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THE EFFECT OF WHOLE BLOOD TRANSFUSIONS ON PLASMA VOLUME RED CELL VOLUME AND EXTRACELLULAR FLUID VOLUME IN PATIENTS WITH ANEMIA *

JAMES D MCMURREY LOYD GALE RIPLEY AND MICHAEL E DE BAKEY

During the course of a considerable number of total body composition studies in cachectic patients it was observed that these patients generally exhibited an absolute and relative reduction in red cell volume with relative increases in plasma volume and extracellular fluid volume Total blood volume generally showed a decrease which was less in proportion to the reduction in body weight[1] Preliminary findings in a few of those patients suggested changes in several aspects of the body composition with correction of the red cell deficit which these patients manifested It seemed of interest to investigate further

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* From the Cora and Webb Mading Department of Surgery Baylor University College of Medicine Houston Texas Supported in part by the Department of the Army Research and Development Division through Contract No DA 49 007 MD 868 and Contract No DA 49 007 MD 564

the changes in some of the body constituents during the process of correction of anemia in patients with chronic blood loss This type of patient would also offer the opportunity to study further the concept of chronic shock as well as the usual method of correction of this abnormality namely by the administration of whole blood It was anticipated that this group of patients would manifest many of the abnormal changes in body composition characteristic of chronic illness and that the magnitude of change between two determinations separated by the administration of whole blood would be gross enough to detect by methods currently available It was the further intent of this study to observe the time factor necessary for readjustment after correction of anemia since in clinical practice it has been customary to allow a period of one to two days after administration of multiple whole blood transfusions to avoid circulatory overloading in the immediate presurgical period

METHOD

Seven patients were selected for this study who showed evidence of anemia as determined by low venous hematocrit In 6 patients history and laboratory findings were consistent with chronic blood loss and in one patient the chronic anemia was apparently due to chronic sepsis Serial body weights were obtained using a bed scale and determinations of red cell volume, using chromium [51] tagged washed red blood cells plasma volume using Evans blue dye and extracellular water volume using bromide [82] were carried out as soon as feasible after admission of the patient to the hospital These determinations were repeated after administration of whole blood in amounts greater than 1000 cc with a time interval ranging between one and ten days between the last blood transfusion and the second determination In one patient H J three sets of determinations were carried out with an interval of two days separating the first set of transfusions and the second determination and an interval of four days separating the second set of transfusions and the third determination

Table 1 Alterations of Body Composition with Whole Blood Transfusions One to Two Day Interval for Readjustment

Patient	Transfusion Volume (ml) Interval after Transfusion	Date of Study	Weight (kg)	Hematocrit (%)	Blood Volume (ml)	Red Cell Volume (ml)	Plasma Volume (ml)	Extracellular Water Volume (L)
E D	1000	3/24/58	49.2	21	5710	715	4995	19.2
	1 Day	3/28/58	50.8	28	4940	840	4100	19.9
T C	1500	3/24/58	54.0	28.5	4[illegible]60	1100	3660	15.6
	1 Day	3/28/58	54.4	33.0	5720	1360	4360	17.5
H J	1500	7/7/58	54.1	25.5	4560	815	3740	22.3
	2 Days	7/10/58	54.5	31.0	4455	1160	3290	27.2
B J	1500	8/14/58	59.0	26.0	3070	609	2110	11.9
	2 Days	8/18/58	59.1	42.5	3360	1100	2260	12.2

Table 2 Alterations of Body Composition with Whole Blood Transfusions Four to Ten Day Interval for Readjustment

PATIENT	TRANSFUSION VOLUME (ML) INTERVAL AFTER TRANSFUSION	DATE OF STUDY	WEIGHT (KG)	HEMATOCRIT (%)	BLOOD VOLUME (ML)	RED CELL VOLUME (ML)	PLASMA VOLUME (ML)	EXTRACELLULAR WATER VOLUME (L)
J M	1500	2/28/58	87 9	27	6905	1315	5590	21 5
	5 Days	3/6/58	86 3	36	6045	1465	4580	18 0
T H	1000	2/28/58	71 6	34	6530	1590	4940	23 9
	4 Days	3/6/58	67 4	41	6780	1910	4870	23 0
H J	1500	7/10/58	54 5	31	4455	1160	3290	27 2
	4 Days	7/17/58	54 2	36	4120	1525	2600	—
A B	1500	8/11/58	57 6	19 5	5660	875	4785	21 7
	10 Days	8/26/58	50 8	33	4750	1060	3700	17 2

RESULTS

These patients were divided into two groups the first having an interval of 2 days or less between the last blood transfusion and the time of subsequent study, and the second having a time interval of from 4 to 10 days between the last blood transfusion and the subsequent study The findings in those patients with the short time interval are listed in Table 1, and those patients with a longer time interval are listed in Table 2 After the administration of 1000 to 1500 ml of whole blood, with a time interval of 1 to 2 days all patients gained weight, with the increase ranging between 0 1 kg to 1 6 kg An increase in hematocrit of from 4 5% to 16 5% was observed Similarly the red cell volume in all patients was increased, with the increment ranging between 120 ml and 495 ml In this group of patients, the extracellular fluid volume also increased with an increment ranging between 310 ml and 4900 ml The changes in blood volume and plasma volume were not uniform In some patients an increase and in some patients a decrease in each of these quantities was noted

In the group of patients with a time interval of from 4 to 10 days between transfusion and second determination a decrease in weight of from 0 3 kg to 6 8 kg was noted In all patients hematocrit increased, with the increment of 5% to 13 5% The red cell volume in this group also increased in all patients with the increment ranging between 150 ml and 360 ml All patients in this group underwent a reduction in plasma volume and extracellular fluid volume, with the decrement in plasma volume ranging between 70 ml and 1110 ml, and the decrement in extracellular fluid volume ranging between 885 ml to 4531 ml The response of the blood volume to transfusion was variable with 3 of the 4 patients showing a decrease ranging between 330 ml to 900 ml An increase of 250 ml was noted in one patient

DISCUSSION

The body compositional findings in these patients are in general in accord with the findings seen in chronic cachexia in larger studies of this type It should be emphasized that the selection of patients was based upon the hema

tocrit findings, and that a low hematocrit finding is not necessarily indicative of anemia. In each of the patients studied, a valid reason for anemia was apparent in the diagnosis, and, presumably, these patients are similar to a cross-section of patients where it is felt necessary to correct anemia prior to surgical operations. The findings presented demonstrate a difference in the response of patients to blood transfusion between 1 and 2 day periods and 4 to 10 day periods of time. Though the number of patients presented is not great, the findings would suggest that after whole blood transfusion, there is an initial increase in blood volume which during the period of adjustment is returned to a fairly normal level, with the retention of red blood cells and the discard of plasma. The extracellular water volume is also increased early after blood transfusion, but declines below its former level during the period of readjustment. Weight, likewise, is increased early, but is decreased during the period of adjustment to a level below that prior to the institution of blood transfusion therapy.

Of most interest in this study is the decrease in weight and extracellular fluid volume that is effected by the addition of whole blood. These studies might lend some credence to the concept of a volume receptor, and would tend to suggest that this receptor is sensitive to some aspect of the vascular volume or to the ratio of red blood cells to plasma.

These findings further suggest that the period of adjustment required for body compositional alteration to an optimum level after two or more units of blood are given is in excess of two days, and, probably, on a clinical basis, a period of a week should be allowed for this adjustment. It seems doubtful that readjustments of the type seen in this study would follow massive blood replacement after sudden massive blood loss, since it is probable that a considerable period of time is required to produce alterations in body composition with anemia.

SUMMARY

Seven patients with chronic anemia, as evidenced by low venous hematocrits, were studied from 1 to 10 days following the administration of whole blood, with determinations of red cell volume, plasma volume, and extracellular fluid volume, with a concomitant determination of weight. In the early phase, 1 to 2 days, there is an increase in body weight, an increase in blood volume, an increase in extracellular water, and a variable change in plasma volume. After a period of 4 to 10 days, weight uniformly decreases below that of the initial weight, blood volume undergoes a variable change, and, uniformly, there are increases in red cell volume, with decreases in plasma volume and in extracellular fluid volume.

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Table 2 Alterations of Body Composition with Whole Blood Transf Four to Ten Day Interval for Readjustment

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	5 Days	3/6/58	86 3	36	6015	1465	4580
T H	1000	2/28/58	71 6	34	6530	1590	4940
	4 Days	3/6/58	67 4	41	6780	1910	4870
H J	1500	7/10/58	54 5	31	4455	1160	3290
	4 Days	7/17/58	54.2	36	4120	1525	2600
A B	1500	8/11/58	57 6	19.5	5660	875	4785
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tocrit findings, and that a low hematocrit finding is not necessarily indicative of anemia. In each of the patients studied, a valid reason for anemia was apparent in the diagnosis, and, presumably, these patients are similar to a cross-section of patients where it is felt necessary to correct anemia prior to surgical operations. The findings presented demonstrate a difference in the response of patients to blood transfusion between 1 and 2 day periods and 4 to 10 day periods of time. Though the number of patients presented is not great, the findings would suggest that after whole blood transfusion, there is an initial increase in blood volume which during the period of adjustment is returned to a fairly normal level, with the retention of red blood cells and the discard of plasma. The extracellular water volume is also increased early after blood transfusion, but declines below its former level during the period of readjustment. Weight, likewise, is increased early, but is decreased during the period of adjustment to a level below that prior to the institution of blood transfusion therapy.

Of most interest in this study is the decrease in weight and extracellular fluid volume that is effected by the addition of whole blood. These studies might lend some credence to the concept of a volume receptor, and would tend to suggest that this receptor is sensitive to some aspect of the vascular volume or to the ratio of red blood cells to plasma.

These findings further suggest that the period of adjustment required for body compositional alteration to an optimum level after two or more units of blood are given is in excess of two days, and, probably, on a clinical basis, a period of a week should be allowed for this adjustment. It seems doubtful that readjustments of the type seen in this study would follow massive blood replacement after sudden massive blood loss, since it is probable that a considerable period of time is required to produce alterations in body composition with anemia.

SUMMARY

Seven patients with chronic anemia, as evidenced by low venous hematocrits, were studied from 1 to 10 days following the administration of whole blood, with determinations of red cell volume, plasma volume, and extracellular fluid volume, with a concomitant determination of weight. In the early phase, 1 to 2 days, there is an increase in body weight, an increase in blood volume, an increase in extracellular water, and a variable change in plasma volume. After a period of 4 to 10 days, weight uniformly decreases below that of the initial weight, blood volume undergoes a variable change, and, uniformly, there are increases in red cell volume, with decreases in plasma volume and in extracellular fluid volume.

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THE EFFECTS OF HYDRATION ON EPINEPHRINE INDUCED RENAL SHUTDOWN IN DOGS *

CHARLES R HATCHER, JR, JOHN A GAGNON AND ROBERT W CLARKE

A reproducible experimental method for the production of acute reversible and irreversible renal insufficiency in dogs has recently been reported by the authors In the standardizations of that technique, which produces renal ischemia by a time controlled spasm of the renal vasculature, it was noted that the degree of injury sustained by the kidney was related significantly to the state of hydration, i e the rate of water excretion at the time of insult The importance of dehydration in the development of renal insufficiency associated with hemoglobinuria is well documented [1 2 3] Likewise the protective effect of an osmotic diuresis against a combined insult of renal ischemia and an intravenous infusion of methemoglobin has been demonstrated [4] The relationship between state of hydration and tolerance of renal ischemia alone has not been so clearly defined We therefore conducted studies of the effects of a standard ischemic insult in dogs in various states of hydration dehydration or mannitol diuresis

METHOD

The 30 mongrel female dogs employed in this study weighed 10 to 20 kg and were obtained from kennel stock Baseline 24 hour urine volumes were recorded and blood urea nitrogen determinations were made on one or more days prior to the experiment Two groups of dehydrated animals received nothing by mouth 24 and 48 hours prior to the infusion of epinephrine The animals of the hydrated group were studied in pairs One animal of each pair received normal kennel rations and water *ad lib* until the morning of the experiment One to two hours before being anesthetized, tap water (50 ml /kg) was given by gastric tube The other member of the pair had an identical water load plus 12 5 gm of mannitol intravenously immediately after anesthesia At the time the water load was administered, a Foley catheter was placed in the bladder and subsequent urine flow was recorded Anesthesia was by pentobarbital, 30 mg /kg Under sterile operating room conditions a right nephrectomy was performed through a midline incision The left renal artery was then exposed near its point of origin from the aorta, and a curved 27 gauge needle shaft attached to a fine polyethylene tube was introduced into the renal artery Infusion of USP adrenalin (epinephrine/norepinephrine), 10 μg/minute in a volume of 0 3 ml of 5% dextrose in water was begun promptly and maintained at a constant rate with a pump for 1 or 1¼ hours Bladder washout with water and air was done periodically throughout the experiment At the conclusion of the period of infusion the needle was withdrawn and pressure applied to the artery to prevent bleeding The abdomen was closed in layers Penicillin and streptomycin were given for at least 4 days postoperatively Daily body weight 24 hour urine volume, urinalysis BUN and hematocrits were noted until the time of death or sacrifice Serum sodium, bicarbonate and potassium concentrations were measured when in

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dicated. Daily intake consisted of 50 ml. of 50% dextrose in water by vein until recovery permitted a low protein kennel ration mixed with measured amounts of water. Fluid loss from vomiting or diarrhea was replaced with isotonic saline and acidosis was adjusted with sodium bicarbonate.

RESULTS

Six of the 7 animals, deprived of water for 48 hours prior to one hour of renal arterial infusion of epinephrine, died of acute renal failure, typically in 10 days (Figure 1). The BUN rose to extremely high values and although the daily urine output returned to normal levels the specific gravity remained fixed at about 1.012 prior to death. The rapidly rising urine production curve of the figure is due more to a reduced output before infusion due to dehydration than to a postinfusion diuresis. The one animal surviving this renal insult was a borderline case for several weeks during which the BUN rose to 70 mg.% by day 7 and then started a very slow decline toward normal. However, the ability to concentrate urine above 1.015 when stressed with pitressin or a water deficit was lost for nearly three months following insult.

In the second group of 8 animals deprived of water for only 24 hours, 7 survived following the same period of renal infusion (Figure 2). The daily urine output was normal and the specific gravity, which was low on the first day postinfusion, returned to normal concentrations. The average BUN rose to 50 mg.% by day 4 and then fell gradually prior to sacrificing on day 7. The one nonsurvivor in this group died of acute renal failure on the fifth day.

When 5 additional animals were deprived of water for 24 hours just prior to a more prolonged period of infusion (1¼ hr), only one animal survived, the other 4 dying renal deaths within an average of 7 days (Fig. 3). The BUN rose to over 200 mg.% and although the daily urine output was adequate in volume, it was of a low specific gravity. The one survivor in this group could also be considered a borderline case as the BUN rose to 75 mg.% by the second day and didn't return to normal for 2 months. During the 3 month period following renal infusion it lost and regained its power to concentrate several times.

Five normally hydrated animals, similarly infused for 1¼ hours and given a water load (50 ml./kg.) 2 to 3 hours before infusion, all died a renal death on the average of 8 days (Figure 4). Although the BUN rose to very high values and the power to excrete a concentrated urine was lost, the daily urine output had increased to a respectable level prior to death.

In the group of normally hydrated animals infused for 1¼ hours in which a water load plus mannitol was administered prior to infusion, 4 of the 5 animals survived (Figure 5). Unlike the water loaded animals which did not receive mannitol, [illegible] the first 24 hours following infusi[illegible] hen gradually returned to norma[illegible] 2nd day and then returned to normal before the dogs were sacrificed. The specific gravity was low on day one as might be expected due to the excreted water load but subsequently returned to levels above 1.020.

In an effort to determine the duration of postinfusion anuria, urine output was measured for an additional 5 hours following infusion on the 10 animals given mannitol and/or water (Table 1). Those animals receiving only a water load excreted a maximum of 2 ml. of urine during the first 5 hours and died,

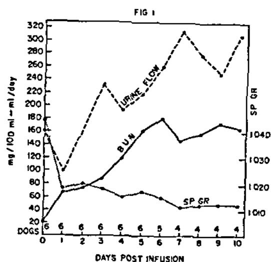


Fig 1 Average data from 6 dogs deprived of water for 18 hours prior to 60 minutes of intrarenal arterial infusion of epinephrine

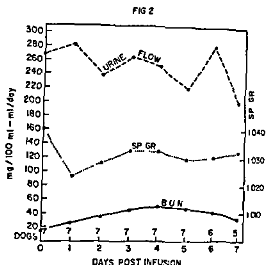


Fig 2 Average data from 7 dogs deprived of water for 24 hours prior to 60 minutes of intrarenal arterial infusion of epinephrine

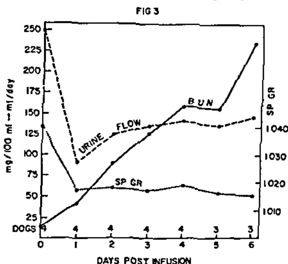


Fig 3 Average data from 4 dogs deprived of water for 24 hours prior to 75 minutes of intrarenal arterial infusion of epinephrine

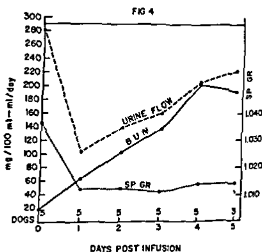


Fig 4 Average data from 5 dogs prehydrated prior to 75 minutes of intrarenal arterial infusion of epinephrine

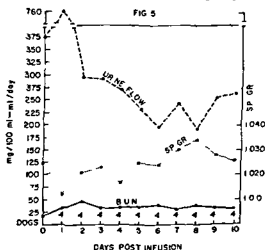


Fig 5 Average data from 4 dogs prehydrated and given 12.5 grams of mannitol prior to 75 minutes of intrarenal arterial infusion of epinephrine

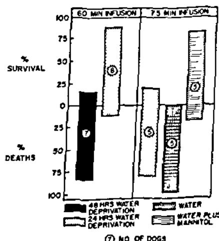


Fig 6 Survival following periods of renal *ischemia in* dogs in various states of hydration or mannitol diuresis

Table 1 Normally hydrated dogs given additional water or water plus mannitol before perfusion

DOG #	HYDRATION—INFUSION INTERVAL (HR)	URINE FLOW PRIOR TO INFUSION (ML/MIN)	URINE EXCRETED 5 HRS POST INFUSION (ML)	DAY OF DEATH AFTER INFUSION
		WATER ONLY		
1	2½	2.7	0	5
2	3	0.3	0.5	6
3	2	0.4	0.5	5
4	2	0.4	0	9
5	1½	0.1	1.0	14
		WATER AND MANNITOL		
6	2	4.1	56	(survived)
7	1½	1.6	31	"
8	2½	3.1	110	
9	2¼	1.5	186	
10	2¼	2.3	2	7

typically in 5 days, of acute renal failure When given a water plus mannitol load, however, 4 of the 5 animals excreted from 31 to 186 ml of urine during this 5 hour period and were permanent survivors The one death in this group excreted only 2 ml of urine in the 5 hour period following infusion and died 7 days postoperatively of acute renal failure

DISCUSSION

Preoperative planning for anesthesia and surgery normally requires a limited or absent oral intake for 8 to 12 hours prior to the procedure During this time intravenous fluids if given are employed sparingly, hence the patient who sustains an episode of renal ischemia during surgery does so during a period of diminished water excretion This state of oliguria, superimposed upon a period of renal ischemia, may then be misinterpreted as acute renal failure with *fluid and electrolyte therapy then directed toward the prevention* of edema The resulting contracted plasma volume, even in the absence of hemorrhage, may be sufficient in itself to lead to a compensatory selective vasoconstriction in which the renal vasculature participates [5, 6]

In this study we have shown that renal functional impairment and mortality following standard episodes of renal ischemia appear to be related not only to the state of hydration but also to the rate of urine flow or rate of water excretion immediately after the renal insult and also to the degree of renal ischemia (Figure 6)

Although it appears as though there is no correlation between survival and normal hydration following severe bouts of renal ischemia there does appear to be good correlation between survival and state of hydration following shorter periods of ischemic insult Other observers also have found that a state of good hydration is beneficial in preventing irreversible renal fail

ure [7 8 9 10 11] The state of normal hydration alone however is not enough to prevent oliguria or anuria following the more prolonged periods of ischemia for in those normally hydrated animals in which a mannitol diuresis was induced there was an incidence of high survival while those animals in a similar state of hydration which did not receive mannitol all died of acute anuria following the same period of ischemia

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AN ION EXCHANGE RESIN ARTIFICIAL KIDNEY *

DAVID CHAS SCHECHTER THOMAS FRANCIS NEALON JR AND
JOHN HEYSHAM GIBBON JR

Kolff and Berk [5] in 1913 demonstrated the feasibility of using an extracorporeal device clinically to lower the levels of serum constituents approaching lethal concentration as a result of renal shutdown They used hemodialysis across a cellophane membrane This apparatus and modifications of it have had widespread clinical acceptance It probably had its peak usage during the Korean conflict Although effective hemodialysis is time consuming and necessitates several trained individuals for its performance The equipment is elaborate and expensive and hence is available only in a limited number of hospitals

In 1956 Kolff stated that the future for the resin artificial kidney is practically unlimited and almost unexplored We have attempted to develop a simple and inexpensive artificial kidney based on the properties of ion exchange resins to lower elevated potassium levels

METHOD

The apparatus † is constructed in its entirety of vinylite a hemorepellent polyvinyl plastic which may be readily sterilized by autoclaving The resin

† Manufactured to the authors specifications by Fenwal Laboratories Somerville N J

* From The Jefferson Medical College of Philadelphia Supported in part by a grant from the United States Public Health Service (H 3349)

employed is Dowex 50-X8 a sulfonated aromatic hydrocarbon polymer cation-exchange resin in the sodium cycle. It consists of nonhemolytic,[7] insoluble beads of 50 mesh size. The resin is encased in seamless columns, 12 cm. long, 2.8 cm. in diameter, and 0.2 cm. thick wall, and containing 50 gm. of resin supported on nylon bolting cloth filters of 100 mesh size.

The inflow (influent) limb consists of a 15 gauge laminar flow needle attached to a Y connector, one arm of which is employed for sampling (Figure 1A). The other arm leads to another Y connector equipped with adapters to which the resin columns are attached in parallel. The outflow (effluent) system is similar to the inflow limb. The incorporation of a plastic container of known capacity (Figure 1B) enables accurate determination of blood flow through the apparatus.

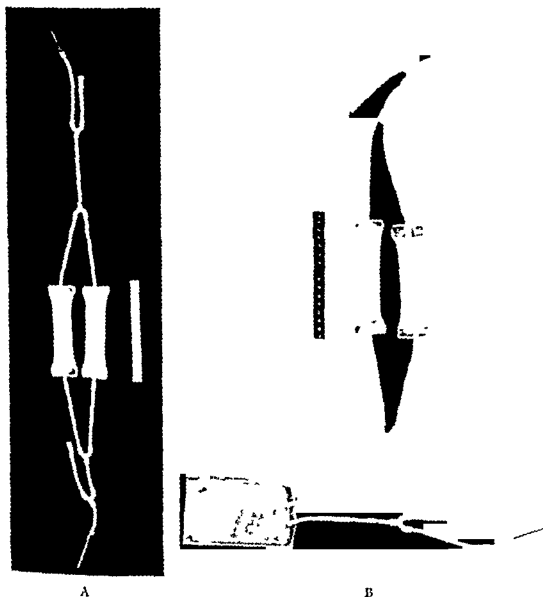


Fig. 1. The ion exchange resin artificial kidney. In "A" the influent and effluent limbs are identical. The incorporation of a plastic container of known capacity in the effluent system "B" enables determination of blood flow through the apparatus.

METHOD

Twelve adult mongrel dogs were subjected to a one stage bilateral nephrectomy After recovering from the operation, 5 were observed untreated while the other 7 were treated with the artificial kidney In the latter group, anesthesia was induced and maintained with minimal doses of intravenous sodium pentothal Two hundred units of sodium heparin per kg body weight were administered The needle of the influent tube was inserted into the femoral artery, and blood was allowed to fill the entire apparatus Less than 30 ml of blood was required The effluent limb needle was then inserted into a femoral vein and hemoperfusion was started Fresh resin columns were substituted every 15 minutes on an empiric basis In order to maintain an uninterrupted stream of blood in the circuit, replacement of the columns was performed alternately as follows clamps were applied immediately proximal and distal to a column The latter was disconnected and discarded A fresh column was attached to the proximal adapter and allowed to fill with blood following which it was connected to the distal adapter and the adjoining clamp removed to enable blood to circulate Less than 10 ml of blood was lost with each discarded column Hemoperfusion was conducted for at least 60 minutes Blood samples were withdrawn from the influent sidearm initially, at 15 minute intervals during perfusion and 30 minutes following perfusion After the last sample was obtained, the animal was given intravenously an amount of protamine sulfate equivalent to the heparin administered, as well of 20 ml of 10% calcium gluconate, 25 mg per kg of body weight of magnesium sulfate, and 500 mg of oxytetracycline, intramuscularly Plasma potassium concentration in the blood samples was determined by internal flame photometry

RESULTS

The 5 control nephrectomised animals survived 96 96, 93, 92 and 85 hours postoperatively The 7 treated animals were perfused 70 to 96 hours after the bilateral nephrectomy Like Thorn and Gibson[7] we observed no hemolysis from passage of the blood over the resin Four of the animals were sacrificed 3 hours after perfusion There was no gross evidence of bleeding from or injury to, any organs of the body The other 3 dogs were observed until their demise from uremia One of these which was moribund when perfused 96 hours postnephrectomy, lived an additional 18 hours Another perfused 90 hours postnephrectomy, survived an additional 40 hours The third also perfused 90 hours after nephrectomy lived 62 hours after perfusion

Prior to perfusion the potassium concentration in the plasma ranged from 6 3 to 9 5 mEq /L (Figure 2) After perfusion for 60 minutes, the potassium concentration was less than 5 0 mEq /L in 5 of the 7 animals In the other 2, there was a drop from 9 5 to 6 3 and from 8 9 to 5 5 mEq /L

The effect of perfusion on blood urea nitrogen concentration was variable In all instances, there was some decrease, but in none did the level return to normal range The smallest reduction was from 152 to 141 and the largest from 194 to 97 mg per 100 ml of blood

DISCUSSION

Since 1950 following the suggestion of Elkinton and his associates,[2] cation exchange resins have been employed either orally or by enema for the elimination of accumulated extracellular potassium in renal failure The enteric

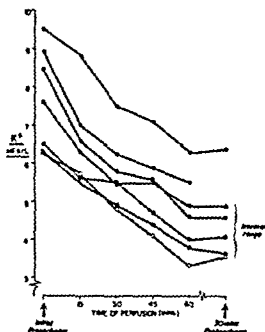


Fig. 2. Reduction of hyperkalemia in uremic dogs by hemoperfusion with the ion exchange resin artificial kidney

route of administration is unpredictable and poorly tolerated. Apparatus for perfusion of blood directly across resins have been constructed by Muirhead and Reid,[6] DeMarchi and Bronnimann,[1] and Kessler, Liebler, Abrahams and Sass.[3] These investigators used glass containers for the resin and encountered problems with sterility, pyrogenicity, hemolysis, solubility of resin and with the lack of satisfactory means for rapidly reconstituting the resin bed. By employing an insoluble and nonhemolytic resin in hemorepellent plastic containers which can be autoclaved, most of these problems have been overcome. Since the columns are disposable, the resin does not have to be reconstituted.

No expensive equipment is required. The total cost of the resin artificial kidney described above is less than the cost of the tubing required in the currently used dialyzing apparatus. Because of its compactness, no blood is needed for priming. Resistance to flow is low enough so that the arterial blood pressure is quite adequate to circulate the blood, and a pump is not required. No voluminous quantities of dialyzing solution need be prepared. The procedure could be carried out at the bedside by a physician with one assistant. If the present apparatus proves to be clinically practical, it could be made available to patients who at present do not have access to treatment with the dialyzing type of artificial kidney.

SUMMARY

1. A simple, compact and inexpensive extracorporeal device for lowering blood potassium by hemoperfusion across an ion exchange resin is described.

2. Dogs rendered uremic by bilateral nephrectomy were treated by this apparatus. Perfusion for one hour resulted in a marked decrease in the plasma potassium.

3. No untoward effects of the perfusion were observed.

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GASTRODIALYSIS WITH A SEMI PERMEABLE MEMBRANE BALLOON APPLICATION TO RENAL FAILURE AND OTHER METABOLIC DERANGEMENTS *

PAUL R SCHLOERB

Many clinical disorders including renal failure congestive heart failure and postoperative fluid electrolyte derangements are in part related to excesses of water or electrolytes or both Although transfer of water across the gastric mucosa is rapid because of rapid reabsorption the net secretion is relatively small Aspiration by an indwelling gastric tube therefore removes only a few liters a day at most A semi permeable membrane bag containing a hypertonic solution should afford a method of removing secreted gastric fluid and electrolytes before reabsorption could occur

It is the purpose of this paper to present an experimental evaluation of this hypothesis and to summarize its preliminary application to the problem of removal of excess body water electrolytes and urea This has been reported previously in abstract form [1]

METHOD

Experiments were done to make a semi permeable membrane bag which would afford maximum dialysis but would be strong flexible and sufficiently resistant to gastric chemical action and peristalsis to permit introduction into the stomach These studies were directed toward an evaluation of the optimum amounts of collodion glycerol ether ethyl alcohol and of the time factors for drying A technique has evolved for making these membrane bags in a few minutes A solution of 6% collodion and 1 2% glycerol in 1 2 ethyl alcohol ether was evaporated on the inside of a 500 ml Kjeldahl flask the neck of which was cut off to about 7 cm After pouring 15 ml of the above solution into the flask it was inverted and was drained into a beaker for one minute with the neck below the surface of the liquid to prevent entrance of air The beaker was then removed and the inner film was allowed to dry for five minutes The film was extracted intact by running cold tap water be

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tween the film and the flask. The bags were stored in moist gauze in the refrigerator. Prior to use, soft, #14 polyvinyl tubing was inserted, and the neck of the bag was tied over a 3 mm. diam. rubber bushing with 2-0 cotton.

Tests were made *in vitro* by filling the bags with 300 ml. of saline and immersing them in a 1 L beaker containing 700 ml. of solution consisting of potassium chloride, 15 mM/L., and urea 400 mg.%. Two milliliter samples were taken from the bag every 15 minutes for 1 hour and were analyzed for potassium and urea nitrogen by standard methods.

The membrane balloon with attached tube was introduced through the nose and was swallowed easily by the human subject. Intubation of bilaterally nephrectomized dogs was accomplished under sodium pentobarbital anesthesia (30 mg. per kg.) with the bag tied on a #18 Levine tube. The balloons and small tubes were swallowed by 3 patients with chronic uremia after spraying the pharynx with 5% cocaine.

The dialyzing solution simulated the electrolyte concentration and pH of gastric juice except for omission of potassium and nitrogen. Hypertonic (50%) glucose or 85% sucrose solutions were used for water removal studies.

RESULTS

Tests *in vitro* showed transfer of potassium and urea into the bag at a rate equal to 30% of the outside concentration in 15 minutes, with stirring of the outside solution.

In the normal human subject, 300 ml. of dialyzing solution, similar to gastric contents but containing no potassium or nitrogen, was infused, aspirated, and replaced each 15 minutes for 2 hours. Removal rates of 0.75 mEq. of potassium and 5.6 mg. of nonprotein nitrogen each 15 minutes were maintained over the 2 hour period, suggesting that the rates of potassium and nitrogen secretion into the stomach were more rapid than the rates of dialysis into the balloon. These rates correspond to the removal of 72 mEq. of potassium and 540 mg. of nonprotein nitrogen per day with continuous infusion-aspiration at 15 minute intervals.

Evaluation studies in bilaterally nephrectomized dogs showed removal of nitrogen in proportion to the blood concentration. In one study, with a plasma NPN of 226 mg.%, nitrogen was removed at a rate of 33 mg./hour while potassium removal was only 0.1 mEq./hour.

Gastrodialysis in one patient, a 19 year male with chronic glomerulonephritis, an NPN of 136 mg.% and a plasma potassium of 5.8 mEq/L. resulted in the removal of 0.6 $\pm$ 0.1 mEq. of potassium/hour and 33.4 $\pm$ 8.2 mg. of nitrogen per hour. Two days later, similar values were observed with the removal of 0.7 mEq. of potassium and 51.3 mg. of nitrogen per hour. In this same patient, 25% and 50% glucose were used for perfusions to remove water, and this resulted in increased potassium and nitrogen removal as well. The amount of water removed was about 21% of the volume of 50% glucose instilled at hourly intervals and 16% of that instilled at 30 minute intervals. With these latter conditions, removal of 94 ml. of water, 140 mg. of nitrogen, 1.5 mEq. of potassium, 9 mEq. of sodium, and 13 mEq. of chloride/hour was achieved in this patient with chronic uremia. Hypotension, sweating, and weakness resembling "dumping syndrome" occurred on one occasion.

Similar studies in another patient, an 18 year male also having chronic glomerulonephritis with an NPN of 176 mg.% showed roughly comparable

results Using 5% glucose in water for gastrodialysis, removal of about 2 mEq of potassium, 24 to 88 mg of nitrogen and 12 to 40 ml of water per hour was found Perfusion with 50% glucose or 85% sucrose resulted in the removal of 1 to 3 mEq of potassium 53 to 144 mg of nitrogen, and 66 to 140 ml of water per hour

A third patient, terminal with severe uremia on the basis of glomerulonephritis had ten gastrodialyses over a period of about 12 hours Removal of potassium at a rate of about 0 5 mEq /hour and non protein nitrogen at 97 mg /hour was effected Although the sensorium cleared temporarily, it was not possible to relate this exclusively to the gastrodialysis because of variable respiratory factors before and during the procedure

DISCUSSION

Transfers of H^+, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, water, and nonprotein nitrogen across a collodion glycerine membrane balloon within the stomach have been shown to occur Placement of the balloon by swallowing or intubation has not been attended by complications or difficulty

Removal of potassium and nitrogen was relatively slow due to the limited surface area (300 cm^2) of the balloon and because of this, removal of sufficient potassium, nitrogen and probably other unmeasured uremic metabolites to substitute totally for renal function would not be possible, but prevention of severe hyperkalemia may be feasible

Electrolyte and hydrogen transfers suggest its application to the management of electrolyte derangements, while selective removal of water at a rate of 2 L /day may have application to the treatment of hyperhydration disorders Further evaluation is continuing

SUMMARY

Gastrodialysis with a collodion glycerine semipermeable membrane balloon is being evaluated Selective removal of electrolytes and water suggest its application to electrolyte derangements and hyperhydration disorders

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ALDOSTERONE SECRETION FOLLOWING SURGERY: CORRELATIONS WITH THE URINARY Na^+/K^+ RATIO*

LOUIS L. SILVA, JAMES STEEL, JEWELL SLOCUM AND JAMES D. HARDY

Despite the well known physiological alteration of the urinary electrolytes in response to surgical trauma there is a paucity of information concerning the relationship of aldosterone to this phenomenon. In the work thus far reported aldosterone determinations have been done on crude[1,2] or partially purified[3] extracts by bioassay methods. Such estimations without prior chromatographic isolation measure aldosterone-like activity which may or may not be closely related to the true aldosterone output or excretion. The present investigation was undertaken to determine the aldosterone output by a chemical method pre and postoperatively and to correlate this with the Na^+/K^+ ratio.

METHOD

Studies were made on 8 patients who were subjected to operations of various degrees of severity. Twenty four hour urine samples were collected for 2 to 4 days prior to and 2 to 7 days following surgery. The unpreserved urine samples were stored in a cold room at 5°C and processed as soon as possible after collection. The extraction and purification of the extract was conducted essentially by the method of Ayres *et al*.[4] The aldosterone was isolated from the residues of the extracts by two stage paper chromatography using chloroform formamide and Bush's system c essentially as described by Neher and Wettstein.[5] Quantitative estimation of the isolated aldosterone was made using blue tetrazolium.[6] The sodium and potassium determinations were carried out using the standard procedure for the Beckman direct reading flame photometer.

RESULTS

The results for aldosterone excretion are expressed in micrograms/gram of creatinine because of occasional incomplete collections of urine. Normal values obtained in this laboratory were: male 19.9 ± 8.0 µg/24 hours or 13.9 ± 8.1 µg/gm of creatinine and female 17.2 ± 6.1 µg/24 hours or 14.9 ± 5.2 µg/gm of creatinine. The average results of aldosterone excretion and the corresponding Na^+/K^+ ratios are contained in Table 1. The first specimen represents the baseline and is the first specimen collected for analysis following admission, in each case collected 2 to 4 days prior to surgery.

DISCUSSION

Examination of the table reveals two peaks in aldosterone excretion, one on the first day preoperatively and the second on the first postoperative day. The rise on the first preoperative day is statistically significant. The possible explanation for this phenomonen is found in the reports of Venning *et al*.[8] who have demonstrated that emotional stress will cause a rise in the urinary aldosterone excretion; therefore it is conceivable that this preoperative rise

*From the Departments of Biochemistry and Surgery, University of Mississippi Medical Center, Jackson. Supported in part by grant H 3873 from the National Heart Institute of the National Institutes of Health, Public Health Service and by a grant from the Mississippi Heart Association.

Table 1 Mean Urine Aldosterone Excretion and Na+/K+ Ratio (8 patients)

	1st specimen†	1st day preoperative	Day of surgery	1st day postoperative	2nd day postoperative	3rd day postoperative
Aldosterone Micrograms/gram Creatinine	11 9	19 1	17 8	28 6	20 1	19 8
Na^+/K^+ Ratio	12 0	4 2	4 8	2 0	2 6	2 4

†2 to 4 days prior to surgery

is due to apprehension or acute emotional stress Our series contained patients undergoing the following operations 2 subtotal gastrectomies 1 subtotal gastrectomy and vagotomy, 1 subtotal thyroidectomy 1 radical neck dissection 1 repair of an inguinal hernia, 1 resection of AV fistula of the right carotid and internal jugular, and 1 right colectomy The stress of surgery *per se* presumably does not stimulate aldosterone secretion and excretion because the peak level of excretion did not occur until the first or second postoperative day, in all except one case The high mean level of excretion on the first postoperative day is not statistically significant from the first specimen but the peak excretion for each patient, wherever it might occur, is statistically significant Llaurado [1] and Zimmerman [3] analyzed only one preoperative and one postoperative specimen and concluded that there was a rise in the aldosterone excretion during the first 24 hours following surgery However, this is not necessarily true since the maximum excretion may occur any time within the first 3 days following surgery, returning to preoperative values within 8 to 15 days postoperatively [2] Our work, which was carried out over a longer period of consecutive postoperative days also indicates that the maximum aldosterone rise does generally occur on the first or second day postoperatively Furthermore, it does not appear to be influenced by the nature of the surgical procedure or by the administration of blood or intravenous fluids, prior to or following surgery

CONCLUSIONS

There is a statistically significant rise preoperatively in aldosterone excretion

There is a significant postoperative rise occurring on the second or third day In general, the urinary Na^+/K^+ ratios are inversely related to aldosterone excretion

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THE REGULATION OF ALDOSTERONE OUTPUT SIGNIFICANCE OF POTASSIUM ION *

WALTER H MORAN, JR, J C ROSENBERG, AND BERNARD ZIMMERMANN

Several workers [1 2] have demonstrated an increase in the urinary excretion of the "3 oxo conjugate of aldosterone following surgery Possible mechanisms include the stimulation of the adrenal gland by the secretion of ACTH at time of surgery, by a fall in serum sodium concentration, by a rise in serum potassium concentration, or possibly by shifts within the fluid compartments On the other hand, this whole phenomena might just reflect an altered route of conjugation and excretion In this experiment the effect of a rise in serum potassium concentration upon the adrenal output of aldosterone without a concomitant change in serum sodium concentration or intravascular volume was investigated

METHOD

The right lumboadrenal vein of 20 kg mongrel dogs was cannulated by the method of Hume and Nelson [3] under nembutal anesthesia Each animal was heparinized after the dissection was complete, but just before the adrenal cannula was inserted Cannulae were also placed in the left femoral artery for recording blood pressure, into the left femoral vein for drawing venous samples, into the right femoral vein for transfusion, and through the right jugular vein into the superior vena cava for infusion of the potassium chloride solution (Fig 1) Blood loss due to surgery and withdrawal of samples was replaced promptly with fresh heparinized blood in silicone coated containers Each animal received about 2 5 ml /kg /hr of fluid during the course of the experiment

One hour was allowed to elapse before the experiment was continued An initial adrenal vein sample was obtained before the potassium chloride infusion was begun The infusion was adjusted to raise the serum potassium concentration about 2 mEq /L and was monitored by serum potassium determinations every 15 minutes Adrenal venous samples were obtained every 3 to 4 hours Serum sodium concentration, pH, serum osmolality and hematocrit were also followed The controls were treated in the same manner but received no potassium

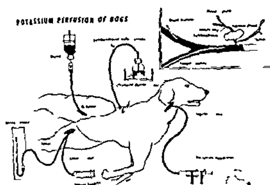


Fig 1

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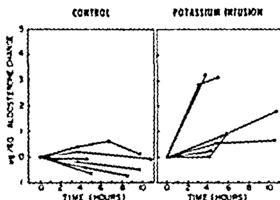


Fig 2 The change in adrenal output of aldosterone in µg /60 minutes

Aldosterone and cortisol were estimated by the following method Three hundred milliliters of adrenal vein blood were collected in oxalated bottles on ice, centrifuged and the plasma frozen for storage A hematocrit was done on the samples The plasma was extracted for 15 minutes with 2 volumes of chloroform three times The combined extracts were filtered, evaporated to dryness and partitioned between 70% methanol hexane twice The methanol fraction was chromatographed for 36 hours in a propylene glycol toluene system [4] The area corresponding to cortisol was eluted and estimated by the blue tetrazolium color reaction in a spectrophotometer The area corresponding to aldosterone was eluted and rechromatographed in an iso, octane tert butanol water system (I $_2$B) [5] The aldosterone was estimated by comparing it visually to both blue tetrazolium reduction and soda fluorescence of 25 .5, 1, 2, 3, 4, and 5 µg cortisol standards run on the same chromatogram [6] Aldosterone is more polar than cortisol Recoveries by this method were 90% for cortisol and 60% for aldosterone Cortisol determinations had a precision of ± 2% in 500 µg range while aldosterone had a precision of ± 30% in 2 to 3 µg range Material isolated from a large pool of dog adrenal vein blood by this method had the same activity as authentic aldosterone when assayed in adrenalectomized rats

RESULTS

A total of 12 dogs, 6 control and 6 potassium infusion, were used in this experiment The average rise in serum potassium was 2 mEq /L and the average rate of infusion of potassium chloride solution was 35 mEq /min There was no significant change in serum sodium concentration, osmolality, hematocrit or venous pH There was no fall in blood pressure The cortisol and aldosterone data are summarized (Table 1 and Fig 2) The adrenal venous flow fell at about the same rate in both infusion and controls during the experiment

DISCUSSION

The increase in aldosterone output following the infusion of potassium was compared to the change in the controls during the same period by the rank sum test" [7] and found to be significant with a probability of 004 The rise noted was not as great as reported by others for hemorrhage, low sodium diet, or inferior vena cava constriction Farrell [8] found no significant increase in aldosterone output when infusing dogs at a constant rate with potassium chloride His experiment was slightly different in that the samples from several dogs were pooled and analyzed as one instead of letting the samples

Table 1. Results of Potassium Infusion and Control Adrenal Venous Samples Analyzed for Aldosterone and Cortisol

	PRE K		POTASSIUM INFUSION DOGS K					
			1		2		3	
DOG	ALDO µg/60′	CORT † µg/60′	ALDO †† µg/60′	CORT µg/60′	ALDO µg/60′	CORT µg/60′	ALDO µg/60′	CORT µg/60′
1	1.8	439	5 0	610	—	—	—	—
2	1 2	—	1 2	—	2 1	548	—	—
3	1.5	266	1.7	222	—	—	—	—
4	2 7	468	4 2	476	4.5	600	—	—
5	1 0	620	1 5	455	1 6	570	—	—
6	0	392	9	264	1.7	216	—	—

	CONTROL DOGS							
	1		2		3		4	
DOG	ALDO µg/60′	CORT. µg/60′	ALDO µg/60′	CORT µg/60′	ALDO µg/60′	CORT µg/60	ALDO µg/60′	CORT µg/60′
1	1 3	263	9	148	6	113	—	—
2	1.0	71	1 4	160	1 6	240	1 1	96
3	9	546	4	178	—	—	—	—
4	1 5	350	1.3	344	1 0	214	—	—
5	.9	315	1.1	290	8	192	—	—
6	1.2	390	1.1	255	—		—	—

† Cortisol
†† Aldosterone

of each dog be analyzed separately. Their rate of infusion was also slightly greater than in our experiment.

The most difficult question is to discern whether the potassium stimulated the adrenal directly or secondarily through some other mechanism such as volume change or ACTH release. The parallel course of cortisol and aldo sterone output suggests the latter. Similar experiments on hypophysectomized dogs are in progress.

SUMMARY

There is no way to be sure that the rise in serum potassium concentration seen following surgery is responsible for the increased urinary output of aldo sterone but it has been demonstrated by this experiment that a rise in serum potassium concentration is capable of increasing the adrenal output of aldo sterone.

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THE USE OF PLASMIN FOR THE INTRAOPERATIVE LYSIS OF CLOTS OCCURRING DURING EXPERIMENTAL ARTERIAL SURGERY *

J. Leonel Villavicencio and Richard Warren

With the availability of purified preparations of human plasmin, attention has been directed toward their use *in* all types of clinical problems involving intravascular clotting both venous and arterial. Most of the observations made in patients treated with human plasmin have been based on indirect evidence of clot lysis. There are few reports in the literature of actual solution of clot *in vivo*.[1, 2, 3, 4] It seemed to us that a more clear-cut preparation was needed in which we could demonstrate the lytic activity of fibrinolysins *in vivo*.

To simulate what happens in human surgery a preparation was selected in which clotting of the blood was produced in a venous autograft anastomosed end to side to the superficial femoral artery (T graft, Fig. 1). This arrangement had the advantage of giving us positive evidence of restoration of continuity if the clot were lysed.

METHOD

Human plasmin prepared from Fraction III of plasma and activated with streptokinase was used.† The contents of 3 to 4 vials containing 25,000 units of plasmin each were dissolved in 350 cc. of 0.85% sodium chloride.

Six adult mongrel dogs in good health (male and female) weighing from 13 to 24 kilos were used.

Analytic Methods. Fibrinolytic activity was determined in all our animals using a combination of three different methods: a) benzoyl arginine methyl ester (BAME) assay in plasma[6] and in the euglobulin fraction of plasma; b) direct observation of lysis of a thrombin fibrinogen clot formed in the presence of the euglobulins of the test plasma;[7] and c) whole blood clot spontaneous lysis time.[8]

Procedure. The dogs were anesthetized with intravenous nembutal and an endotracheal tube placed to secure a free air way. Through an incision at the groin the iliofemoral vessels were exposed and dissected over a length of 10 to 12 cm. A 5 cm. segment of femoral vein was then isolated and removed. An end to-side anastomosis was carried out between the superficial femoral artery and the distal end of the venous graft (T graft, Fig. 1). Precautions were taken to orient the venous valves in the direction of the arterial blood stream.

Once the venous autograft had been anastomosed the femoral artery was

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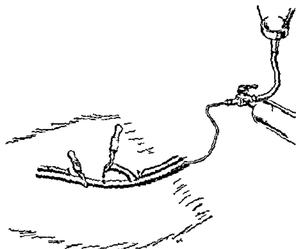


Fig 1 "T" graft Once a clot has been formed in the venous autograft anastomosed to the femoral artery the solution of human fibrinolysins is slowly infused through the external iliac artery Lysis of the clot is attained when blood flows again through the previously clotted graft

cross clamped distal to the graft and a partially constricting "bulldog" clamp placed at the free end of the graft Blood was allowed to drip from the free end at the rate of 35 to 15 drops per minute In about 10 minutes the flow began to slow down, stopping completely in 15 to 25 minutes At the same time, the arterial pulse, which at the beginning of the flow had been easily seen in the venous graft, gradually diminished in amplitude It disappeared completely about 5 minutes after the flow had ceased, indicating that the clot in the graft had involved its whole length

In 1 of the animals the femoral artery was cross clamped with a "bulldog" clamp The obstruction was then completely bypassed, using a 5 cm venous autograft anastomosed end to side above and end to side below A clot was then produced in the graft by clamping it at its two ends[9] and allowing the blood to clot in the isolated segment Five to 10 minutes after the clot had formed the plasmin infusion was started through a fine polyethylene tube inserted 2 cm downwards into the external iliac artery proximal to the graft The rate of infusion was 8 cc /minute Blood loss was replaced with fresh blood given intravenously

When the experiment was considered completed the graft was carefully excised and placed in a test tube at 37°C for observation

RESULTS

***In Vivo* Lysis of Clots in the Venous Autografts.** In all the animals it was observed that blood flow appeared at the narrowed free end of the graft between 11 and 27 minutes after the infusion had been started

In the dog with the bypass graft it was observed that the pulse returned to the segment and the clot that had previously been easily detected by transillumination disappeared in 15 minutes

As shown in Table 1, the amounts of enzyme necessary for the lysis of the clots was between 1286 and 4275 units/kg

***In Vitro* Lysis of Clots Contained in Removed Venous Autografts.** In 4 of 6 grafts removed it was found that clot lysis had been complete In 2 dogs the removed graft contained a soft, fresh clot which had not prevented restoration of flow When these grafts were incubated at 37° and observed, it was seen that the clot became completely lysed in a period of 2 to 4 hours

Secondary Clot Formation In 2 dogs one "T" graft and the "bypass" graft, the plasmin infusion was stopped when the lysis of the clots became evident

Table 1 Use of Plasmin in Intraoperative Clot Lysis (Experimental Observations)

DOG NO	WEIGHT KG	GRAFT TYPE	CLOT FORMATION TIME (MIN.)	CLOT LYSIS TIME (MIN.)	AMOUNT PLASMIN† NEEDED	CLOT LYSIS
1	23	Bypass	18	15	3261	Yes††
2	14.5	T	10	27	3763	Yes
3	11.5	T	20	25	4127	Yes
4	17	T	17	19	4275	Yes††
5	13.2	T	15	11	1836	Yes
6	21	T	20	20	2809	Yes

† Units of plasmin per kilogram

†† Secondary clot formation

Shortly after the infusion was stopped, new clots formed In the "T" graft, 28,500 units of plasmin were necessary for restoration of blood flow and for restoration of expansile arterial pulsations In the 'bypass' graft a small clot was observed in the distal anastomosis It was not possible to detect lysis of it by transillumination, despite the fact that 10,018 units more of plasmin were infused

Bleeding at the Sites of Anastomosis. *Oozing in the Wound.* In 2 dogs oozing of blood appeared in several parts of the operative area and at the anastomosis site, at places in which it had been necessary to apply pressure to control the bleeding when the anastomoses were first performed

Fibrinolytic Activity in Blood Samples Taken During the Procedure. It was observed that the peak of the lytic activity corresponded closely to the moment in which the clot had been lysed and the blood flow restored As was expected, fibrinolytic activity reached its maximum level during the infusion of plasmin and shortly after the plasmin infusion had been stopped

In one dog, however we did not observe a satisfactory fibrinolytic activity in blood at any moment of the study with any one of our methods (bypass graft with secondary clot formation)

DISCUSSION

Lysis of blood clots *in vivo* was clearly demonstrated to occur when human fibrinolysins were administered experimentally to dogs Relatively high doses of enzyme were injected directly into a localized area The enzymatic action was exerted on fresh clots These factors undoubtedly account, at least in part, for the successful results obtained and need to be taken into consideration whenever the use of enzyme is being considered *in clinical cases* High fibrinolytic levels were detected in the blood of 5 of 6 dogs studied These high levels usually reached a peak during the time in which the clot was being lysed We were unable to detect significant fibrinolytic activity for periods longer than 1 hour after the administration of the enzyme However, it is significant that the clots contained in the removed venous autografts lysed completely in 2 to 4 hours after having been taken from the dog's blood stream This means that plasmin continues to act on fibrin, to which it pre

sumably remains adherent for periods longer than those during which it can be detected in the circulating blood

In one dog lysis of the secondary clot did not occur despite a dose of plasmin similar to that used in the other dogs High fibrinolytic levels were not detected in the animal suggesting a correlation between peripheral levels and clot lysis Other factors such as inhibitors (which were not included in our studies) are undoubtedly present in the blood when the enzyme is being administered and could account for the failure to increase levels and for the ineffectiveness of the second plasmin infusion At least during one stage of the plasmin administration however high fibrinolytic levels must have been present in the blood to account for the lysis of the first clot The lability of the fibrinolytic enzyme and the difficulties of detecting it on many occasions have been pointed out by several investigators [10]

The formation of a clot for the second time in 2 dogs reveals that the enzyme does not interfere significantly with the process of coagulation even though lengthening of the clotting time was observed in 2 dogs when high doses of plasmin were used This could explain the oozing observed at the operative area and sites of anastomosis in the two previously mentioned animals

After the clot has been lysed the formation of a new clot is of course undesirable The use of anticoagulants would synergize the action of plasmin and produce the best results in the prevention and treatment of intravascular clotting This has not been tested experimentally

SUMMARY

1 A preparation devised to observe the lysis of blood clots *in vivo* under the action of human fibrinolysis has been described

2 The successful lysis of fresh blood clots produced experimentally in segments of veins anastomosed to arteries and the factors that could account for the lysis of the clots are described and analyzed

3 The possible use of plasmin in the intraoperative lysis of blood clots in reconstructive arterial surgery and the advantage of the concomitant use of anticoagulants are suggested

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A STUDY OF THE PRESSOR EFFECTS OF SEROTONIN AND ITS POSSIBLE ROLE IN MASSIVE THROMBOEMBOLISM *

JOHN W SETTLENBERGER WILLIAM F WEAVER JACOB I FABRIKANT AND WILLIAM G ANLYAN

Although the vasotonic property of serum was known for many years the active principle was not isolated until 1917[1] It was chemically identified as 5 hydroxyindoleacetic acid and termed serotonin Since then exhaustive studies have been carried out to determine the pharmacologic properties of serotonin However, its physiologic role is as yet unknown The only pathologic process in which it has been definitely implicated is the so called malignant carcinoid syndrome It is known that serotonin is released from platelets when blood coagulates *in vitro*[2] and presumably *in vivo*, but no relationship has been demonstrated between it and the clinical manifestations of thromboembolic disease

A relatively small pulmonary embolus may produce catastrophic results In view of this it remains questionable that mechanical obstruction of pulmonary arteries alone is responsible for the physiologic derangements Evidence indicates that vagovagal reflex mechanisms may play a prominent role in producing the signs and symptoms of pulmonary embolism[3, 4] It has been suggested that serotonin released from the embolus may also be a factor as a result of the circulatory changes it causes but this opinion was based on data obtained employing bioassay techniques[5] It is now possible to determine an increased release of serotonin into the blood stream by measuring the urinary excretion of 5 hydroxyindoleacetic acid the metabolic endproduct of serotonin[6]

The purpose of this study was to ascertain whether or not serotonin was released into the blood stream following intravascular thrombosis as measured by the urinary excretion of 5 hydroxyindoleacetic As a corollary the effect of serotonin on the partially blocked pulmonary arterial circuit was also studied as well as the antiserotonin effect of various drugs

METHOD

Thirty five healthy female mongrel dogs were used in this study as follows

Group 1 12 dogs were anesthetized with intravenous nembutal and a slow infusion of saline started in a jugular vein A catheter was introduced into the urinary bladder for 15 minute collections After a control period of 2 hours 2000 units of thrombin in 50 cc of saline was infused over a period of 60 minutes to produce intravascular thrombosis The urinary excretion of 5 hydroxyindoleacetic acid was determined on all 15 minute collections before and for several hours after the thrombin had been administered As a control 6 dogs received saline alone administered at the same rate as the thrombin solution

Group 2 12 dogs were prepared as in Group 1 but received 300 mg of thromboplastin in 50 cc of saline instead of thrombin

Group 3 5 dogs received intravenous nembutal anesthesia A polyethylene

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catheter was introduced into a jugular vein and connected to a slow infusion of saline. An indwelling catheter was introduced into the urinary bladder for 15 minute collections. Under positive pressure anesthesia the chest was opened. Catheters were introduced directly into the main pulmonary artery and the aortic arch and connected to Stathem gauges. Pressures in these vessels were constantly monitored using a twin-viso recording instrument. After a control period of 2 hours an injection of 2 mg. of serotonin was administered intravenously within a period of 5 seconds. This was repeated after the pressures had returned to normal levels. After recovery from the serotonin injections the left pulmonary artery was ligated. Two milligrams of serotonin were again injected into the jugular vein. Subsequent injections of serotonin were in each instance preceded by the administration of 1 mg./kg. of heparin, 25 mg. of chlorpromazine, and 25 mg. of promazine. Five-hydroxyindoleacetic acid determinations were carried out on all of the 15 minute collections of urine.

RESULTS

Group 1: Intravenous thrombin resulted in a significant increase in the urinary levels of 5-hydroxyindoleacetic acid as compared to the controls receiving saline infusions alone (Fig. 1).

Group 2: Thromboplastin also caused an increase in the excretion of 5-hydroxyindoleacetic acid but to a lesser degree than that produced by thrombin (Fig. 2).

Group 3: The intravenous injection of 2 mg. of serotonin resulted in an immediate rise in both pulmonary and aortic blood pressures, the former being more pronounced (Fig. 3). There was an immediate rise in urinary 5-hydroxyindoleacetic acid excretion. Within 3 hours an estimated 25% of the serotonin was recovered in the urine as 5-hydroxyindoleacetic acid (Fig. 4). Ligation of the left pulmonary artery resulted in no alteration of the pulmonary pressure. However, when 2 mg. of serotonin were injected after the artery had been ligated, there was a more exaggerated and sustained pulmonary hypertension than that caused by serotonin alone (Fig. 5). Chlorpromazine and promazine effectively blocked the pressor action of serotonin (Fig 6) whereas the heparin did not (Fig. 7).

COMMENT

Serotonin has been demonstrated in high levels in platelets and is released into the serum of clotted blood *in vitro.* That circulating serotonin levels become elevated in the living animal following massive venous thrombosis is inferred by the increase in the urinary excretion of 5-hydroxyindoleacetic acid following infusion of solutions of thrombin and thromboplastin. As yet, direct measurement of circulating serotonin has not been carried out, but these studies are in progress. In this study it was demonstrated that there is a powerful vasoconstriction of the pulmonary artery of dogs following the intra- [illegible] ...nonstrated to be greater than [illegible] the [illegible] pulmonary artery occurs in the presence of increased blood serotonin [illegible] e resulting pulmonary hypertension is greater and more prolonged than when serotonin acts alone. An increase in serotonin as a result of venous thrombosis may assist in explaining the profound reaction to a relatively small embolus.

It would appear that an antiserotonin agent such as chlorpromazine or

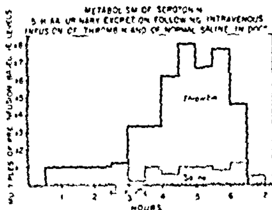


Fig 1 There was a marked increase in the urinary excretion of 5 HIAA following intravenous infusion of thrombin in contrast to normal saline in dogs

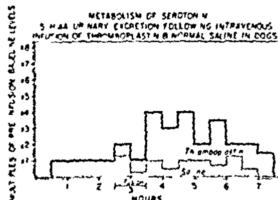


Fig 2 Thromboplastin produced a similar increase in urinary 5 HIAA excretion

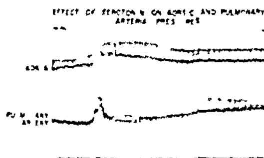


Fig 3 2 mg of serotonin injected intravenously produced a transient aortic and pulmonary arterial hypertension

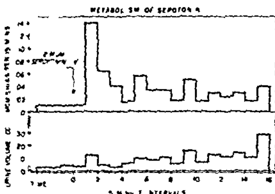


Fig 4 2 mg of serotonin intravenously produced a marked increase in urinary 5 HIAA excretion with 25% recovery in four hours

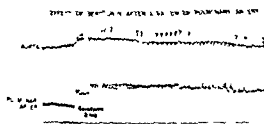


Fig 5 2 mg of serotonin intravenously produced a much more sustained pulmonary arterial hypertension following ligation of the left pulmonary artery

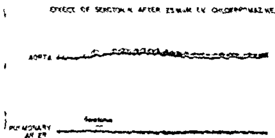


Fig 6 The pressor effect of serotonin was blocked by chlorpromazine

promazine deserves a clinical trial in the treatment of pulmonary embolism This drug is known to lower the urinary 5 hydroxyindoleacetic acid excretion in patients with malignant carcinoid [8] It has no known action on blood coagulation and does not alter an intravascular clot Heparin has been suggested to be beneficial in the treatment of pulmonary embolism because of a direct antiserotonin effect [5] However, such an action was not confirmed in this study It is felt that any clinical response to heparin is a result of its direct

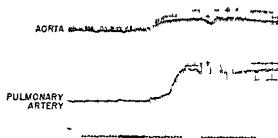


Fig 7 Heparin did not appear to block the pressor effect of intravenous serotonin

action on the venous thrombosis in preventing further propagation of the thrombus

SUMMARY

1 Although direct measurement of free circulating serotonin in the blood has not been carried out, an increase following massive venous thrombosis is indicated by a significant elevation of 5 hydroxyindoleacetic acid urinary levels

2 Serotonin may well be a humoral factor in producing the clinical manifestations of pulmonary embolism not accounted for by mechanical blockage alone

3 Heparin exerts no antiserotonin effect and is beneficial in the treatment of pulmonary embolism only as a result of its anticoagulant action

4 The use of an agent with antiserotonin properties such as chlorpromazine or promazine in the treatment of pulmonary embolism should be investigated

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VARIATIONS IN THE TECHNIQUE OF PLATELET PRESERVATION AT $-79°C$*

WALTER F. BALLINGER II, ARTHUR J. WEISS AND KENNETH M. BLANC

Generalized bleeding following massive transfusions of stored whole blood has been reported on many occasions[1, 2] and is frequently associated with a severe thrombocytopenia[3] The conventional methods of collection and storage of whole blood result in a rapid disappearance of viable platelets[4] Freshly collected platelets have been used in controlling at least temporarily, the hemorrhagic tendency secondary to thrombocytopenia induced by irradiation[5] In addition McGovern[6] recently reported the successful preparation of thrombocytopenic patients for surgery, using transfusions of freshly collected platelets

In recent years the need for a ready source of stored functionally active platelets has become apparent especially since thrombocytopenia is a common cause of death following total body irradiation[7] The use of platelet extracts has been reported in which the hemorrhages in 50% of a small group of children with acute leukemia were temporarily controlled It is our feeling that the use of functionally intact thrombocytes might produce better results We have therefore attempted to utilize the technique of glycerol preservation[8] together with freezing at a temperature of $-79°C$ in order to maintain platelet viability for long periods of time

A previous report[9] by the authors described the apparent viability of platelets preserved by this technique for periods up to 4 months We have since varied the technique of preservation in order to note the effect upon clot retraction this test being considered an excellent reflection of platelet viability *in vitro*

METHOD

Fresh canine blood was collected in plastic containers using a 1 5% solution of disodium ethylene diamine tetra acetate (EDTA) in 0 7% saline as an anticoagulant Following initial centrifugation at 1000 rpm for 7 minutes the resultant platelet rich plasma was centrifuged at 3000 rpm for 30 minutes The platelet mass was washed with isotonic cold saline All centrifugation was performed at 5°C

Samples were then mixed with equal portions of a glycerol saline solution the concentrations of glycerol varying from 0 to 35% All samples containing glycerol were allowed to equilibrate for 4 hours at 4°C following which they were placed in asbestos lined tubes and frozen for 14 hours at $-79°C$ in a methanol dry ice mixture Thawing was accomplished by the rapid immersion of the tubes in a water bath controlled to a temperature of 40°C The samples were then placed in viscose casing and suspended in freshly collected plasma for 2 to 3 hours in order to dialyze the glycerol from the platelet mixture

Some platelet samples were frozen without insulation Other samples were prepared and frozen as described but dialysis was either omitted or varying periods of dialysis were carried out

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RESULTS

Platelet samples containing no glycerol rarely exhibited any evidence of clot retraction following freezing at −79°C When the concentration of glycerol varied between 20 and 30% clot retraction was fair The best and most consistent results were observed when the glycerol concentration was 27% Table 1 lists the representative result for varying concentrations of glycerol

Although not conclusive the addition of an insulating agent such as asbestos appeared to protect the platelets during freezing clot retraction usually being somewhat better in each experiment

The *in vitro* ability of the clot to retract was better in all experiments when the platelet glycerol mixture was subjected to dialysis following freezing and thawing We do not as yet have sufficient data to determine the ideal method of dialysis

Table 1 Effect of Varying Concentrations of Glycerol on the Degree of Clot Retraction

GLYCEROL %	EQUILIBRATION HOURS	INSULATION	FREEZING TEMP	FREEZING HOURS	THAWING TEMP	DIALYSIS HOURS	CLOT RETRACTION
0	0	Asbestos	−79°C	14	40	0	0
10	4	Asbestos	−79°C	14	40	3	0
20	4	Asbestos	−79°C	14	40	2	+
27	4	Asbestos	−79°C	14	40	2	++
30	4	Asbestos	−79°C	14	40	2	++
35	4	Asbestos	−79°C	14	40	2	0
0	0	0	0	0	0	0	++++
20	0	0	0	0	0	1	++
20	0	0	0	0	0	0	0

DISCUSSION

Glycerol serves to protect the cell from the adverse effect of freezing and thawing by binding a considerable quantity of intracellular water preventing hypertonicity and denaturation [10] It has been used extensively in long term preservation of erythrocytes and spermatozoa In the case of erythrocytes the thawed cell containing glycerol is in a state of hyperosmolarity relative to the solution and Sloviter [11] found it necessary to develop a method of dialysis to remove the glycerol gradually

The data presented indicate that the concentration of glycerol is of importance in the protection of platelets during freezing and thawing Under the conditions of equilibration and freezing herein presented a concentration of 27% glycerol appeared to give the uniformly best results

For purpose of *in vitro* investigations of platelet viability dialysis is of importance Whether dialysis of the glycerol from the platelets is required

before infusion into a recepient is not known. The optimal dialysis requirements are currently being investigated

The effect of rapid versus somewhat slower rates of freezing were investigated using various types of insulation materials, such as glass beads, gauze sponges, and asbestos, wrapped around the inner tube containing the platelet-glycerol suspension It was quickly learned that at —79°C only asbestos prevented damage to the plastic tube itself. As noted above, the presence of asbestos appeared to result in somewhat better clot retraction although our data are not conclusive. We are presently attempting to control the rate of freezing more accurately.

CONCLUSIONS

1. Glycerol protects platelets from the adverse effects of freezing at —79°C. and subsequent thawing

2. The optimal concentration of glycerol in these experiments was 27%.

3 For purposes of *in vitro* studies upon platelet function, notably measurement of clot retraction, dialysis of the glycerol from the platelet mass is advisable.

4. Controlled rates of freezing appear to be indicated, since better clot retraction was noted following the use of asbestos insulation.

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PRODUCTION OF LOWER NEPHRON NEPHROSIS IN DOGS BY MEANS OF AN EPISODE OF INTRAVASCULAR CLOTTING *

ROBERT M HARDAWAY AND DONALD G MCKAY

Oliver [1] has suggested the term "ischemuric episode as a substitute for lower nephron nephrosis' because the new term indicates the generally accepted concept that the syndrome, be it a sequel to trauma, transfusion reaction or other disease, is caused by a sudden, transient but profound renal ischemia Shock, with associated spasm of precapillary arterioles of the kidney, has seemed a likely explanation for the ischemia Shock alone, however, does not seem to be the final answer because (a) shock does not always precede anuria,[2] and (b) shock is not usually followed by anuria After studying cases of acute renal failure occurring in Korean War casualties, Teschan [3] reported that "Hypotension alone cannot be incriminated as a cause of renal failure It is the purpose of this paper to present evidence that intravascular thrombi are the cause of the renal ischemia

One of the many conditions associated with the onset of renal failure is an incompatible blood transfusion reaction It has long been known that a transfusion reaction is associated with a bleeding tendency [4] but the nature of the clotting defect remained unknown In 1954 we reported 4 cases of lower nephron nephrosis due to transfusion reaction and associated with a hemorrhagic diathesis [2] In the only one of these cases studied for fibrinogen level there was a complete absence of circulating fibrinogen The hemorrhagic diathesis responded immediately to the administration of fibrinogen It was postulated [2] that the afibrinogenemia was due to the using up of fibrinogen in an intravascular clotting episode and that the necrosis of renal tubules was secondary to intravascular thrombi

In 1955 we first attempted to produce lower nephron nephrosis in dogs by means of an incompatible transfusion reaction [5] The intravenous injection of human blood resulted in a precipitous drop in platelets, a decrease in



Fig 1 Lung showing thrombus in small pulmonary artery

* From the U S Army Hospital Fort Belvoir, Virginia and the U S Army Hospital Frankfurt Germany



Fig 2 Kidney showing thrombi in glomular capillaries

fibrinogen, a prolonged coagulation time and the appearance of a heparin-like anticoagulant. Intravascular clotting also resulted.[6] When the blood was injected rapidly, animals died instantly with numerous small thrombi, predominantly in the lungs (Fig. 1). When the blood was injected slowly the animals survived or died hours later with a few residual thrombi in the lungs and gastrointestinal mucosa. None of these animals developed anuria.

In a further effort to produce lower nephron nephrosis, blood was introduced directly into the aorta above the renal arteries *via* a femoral artery catheter.[7] It was brought that this might concentrate the thrombi more in the kidneys. When only this procedure was done, the dogs survived. In contrast to the intravenous injection which produced thrombi primarily in the lungs, the intraaortic injection produced thrombi primarily in the abdominal viscera, but also in the lungs. Thrombi formation was then studied in 7 dogs by giving the injection with the abdomen open, and taking kidney biopsies every 15 minutes for 3 hours. Other organs were studied at autopsy. It was found that many renal thrombi formed almost instantly but tended to decrease in number in the later biospsies. *All* these animals died in less than 24 hours in deep shock Some of them had a bloody diarrhea All were anuric. At autopsy there was superficial hemorrhagic necrosis of the mucosa of the bowel with pseudomembrane formation and microscopic capillary thrombosis of the bowel mucosa and kidneys (Fig. 2) These dogs resembled clinical cases of pseudomembranous enterocolitis (Fig 3).[8] As these fatal results occurred only



Fig 3 Ileum showing superficial necrosis and hemorrhage on the left with normal mucosa on the right There is a capillary thrombus in the mucosa of the hemorrhagic area

in those experiments with laparotomy and renal biopsy, it was theorized that the trauma caused a certain amount of compensated shock with dilatation of the splanchnic capillaries and slowing of the blood flow therein When to this stagnant blood was added a clotting agent, clotting quickly occurred This caused localization of the thrombi in the bowel As these dogs were all anuric till they died and showed thrombi in the kidneys, it was thought that if they survived longer they would possibly develop lower nephron nephrosis Since the thrombi had appeared almost instantly, there was possibility of protecting the bowel from the fatal hemorrhagic necrosis by occluding the superior mesenteric artery during the transfusion The present paper is a report on the results of experiments designed to test this hypothesis

METHOD

Seventy five mongrel dogs were anesthetized with sodium pentobarbital A laparotomy was performed Both femoral arteries were exposed and a polyethylene catheter inserted in each until the tip reached the level of the diaphragm One catheter was used to inject 4 cc per pound of dog of incompatible (human) blood and the other used to record arterial pressure [9] During the intraaortic injection (5 to 10 minutes) the superior mesenteric artery was pinched off and released on completion of the injection Biopsies of the left kidney were taken at intervals of 15, 30, 60, and 120 minutes after the transfusion and at 3 hours the left kidney was removed

Several variations of this experiment were performed In Group A (11 dogs) the left kidney was freed up before the transfusion so that it was attached only by its artery, vein and ureter

To test the possible effect of the freeing up of a kidney, another group (Group B, 12 dogs) was done in which the kidney was not freed up until after the transfusion was given Otherwise the procedure was the same as in Group A

In all subsequent groups the kidney was not freed up till after the transfusion

To test the effect of acidosis and/or a strongly acid urine, a third group (Group C, 18 dogs) was acidified by various means before the experiment Several other groups totaling 34 dogs had the basic procedure carried out plus dehydration hyperpyrexia or various other situations sometimes associated with the onset of acute renal failure

RESULTS

Group A (With left kidney freed up before injection) Of the 11 dogs 8 developed clinical and pathological findings consistent with a diagnosis of lower nephron nephrosis (Fig 4) Six of these died after 4 to 5 days of anuria with BUNs ranging (with 1 exception) from 120 to 210 mg % The other 2 of the 8 were anuric for a time and developed BUNs of 88 and 112 mg %, but then started to put out urine and seemed on the way to recovery when they were sacrificed Of the remaining 3, one died in less than 24 hours and the other 2 developed only a transient anuria One dog showed microscopic evidence of acute pancreatitis

Group B (With kidney not freed up before injection) Of the 12 dogs, 2 died in less than 24 hours, one died later and the other 9 survived None developed renal failure

Group C (Acidified) Of the 18 dogs, 3 died in less than 24 hours. Nine died later and 6 survived. None developed renal failure.

Of the 31 other dogs, 13 died in less than 24 hours, 2 died later and the 19 others survived with few symptoms None developed renal failure.

DISCUSSION

The fact that the only dogs to develop lower nephron nephrosis had had a kidney freed up before the transfusion was given, whereas not one of 61 other dogs did, points strongly to the fact that trauma adjacent to the kidney must play an important role in the development of renal shutdown. It is remembered that in the earlier experiments, kidney biopsy was necessary to produce fatal bowel lesions. The mechanism of this action is unknown and will be the subject of future investigation Possibly the destruction of autonomic nerve fibers by the act of freeing up a kidney slows the capillary blood flow in kidneys and other abdominal viscera so that thrombi form more easily or persist longer. It is notable in this regard that studies of Korean War casualties by Teschan [3] showed that there was a much higher incidence of renal failure with wounds involving the abdomen and especially the kidney, than with non abdominal wounds, even though the non abdominal wounds were severe enough to be fatal.

SUMMARY

1. It is possible to reproduce the clinical and pathological picture of lower nephron nephrosis in dogs by means of an intraaortic injection of incompatible (human) blood when first a laparotomy is done and a kidney is freed from its bed.

2 To prevent death in shock with a hemorrhagic necrosis of the bowel, it is necessary to protect the bowel from thrombi formation by occluding the superior mesenteric artery during the injection.

3 Acid urine, electrolyte imbalance or dehydration, are not factors in the production of lower nephron nephrosis by this manner in the dog.

CONCLUSION

Intracapillary thrombi are a cause of renal ischemia which will result in death from renal failure and a pathological picture of lower nephron nephrosis

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THE EFFECTS OF HEPARIN AND COUMARIN DERIVATIVES ON A STANDARDIZED INTRAVENOUS THROMBOSIS *

ROGER D. WILLIAMS AND DAN W. ELLIOTT

A method of producing a relatively standard degree of venous thrombosis in one hour has been developed and reported recently.[1] Five millamperes of electric current applied to the external jugular vein of dogs always produced thrombosis. This is a report of the effects of heparin and three coumarin derivatives on thrombosis produced by this new method.

This study was stimulated by the differences of opinion about the relative clinical value of various anticoagulants. Reports of methods testing anticoagulants in experimental animals have also shown conflicting results.[2, 3, 4, 5] Tissue damage, blood stasis, and the degree of thrombosis have been inconsistent. Thrombosis did not occur in all controls. The effects of anticoagulant were not known during the entire experiments. A careful laboratory re-evaluation of anticoagulants is needed.

METHOD

In all experiments 5 ma. of electric current were applied to the external jugular vein(s) for 1 hour. Mongrel dogs weighing from 12.7 to 20 kg. were used. Each vein was carefully exposed. Platinum electrodes 4 by 10 mm. in size, housed in semicircular lucite cuffs, were passed around the vein. The vein wall always made contact with both electrodes. If the vein was occluded by pressure superior to the electrodes, it immediately collapsed. Thus minimal blood stasis occurred.

Heparin was given intravenously in varying doses. It was injected after placing the electrodes but 10 minutes before the electrice current was applied. Initially 50 mg. was empirically given.[1] The clotting time measured by the Lee and White method[6] ranged from 1½ to over 4 hours. In these more recent experiments 0.75 mg./kg. was given. The clotting time varied from 1½ to 3½ times the pre-heparin value (range 3 to 9.5 minutes). It returned to the pre-heparin value 1 to 1½ hours later.

Three coumarin derivatives—dicumarol, tromexan, and coumadin—were given for 2 to 8 days. Large initial doses were used: tromexan 75 mg., coumadin 10 mg., dicumarol 75 to 150 mg. Subsequent daily dosage was usually one half the initial dose except with tromexan which was continued for 2 days at 75 mg. The prothrombin time was determined within 2 hours of each experiment.

The prothrombin time was determined by the method of Quick[7] using Simplastin (Warner and Chillcott) in whole plasma. The value of 24 normal dogs ranged from 8.3 to 9.6 seconds. Pooled dogs' plasma gave a prothrombin time of 8.5 seconds. A prothrombin activity curve, a composite of six plasma samples determined separately in serial dilutions, is shown in Figure 1. A prothrombin time in excess of 15 seconds (18%) was obtained before applying the electrodes in all experiments with coumarin derivatives.

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Supported in part by a research grant H-2513(C1) from the National Heart Institute, Public Health Service.

PROTHROMBIN ACTIVITY CURVE

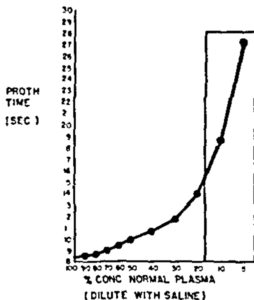


Fig 1 A prothrombin activity curve plotted as a mean from dilutions of six normal canine plasma samples A value within the shaded area (less than 18%) was obtained before thrombosis was attempted in the coumarin experiments

RESULTS

The results are shown in Table 1 In 21 experiments in which no anticoagulant was used a thrombus always formed It formed at the site of maximum vein wall damage which was beneath the positive electrode The thrombus varied from 3 by 9 to 5 by 15 mm in size (averaged 12 by 4 mm) These are controls

Table 1

ANTICOAGULANT	NO EXP	NO CLOTS	SMALLEST CLOT (mm)	LARGEST CLOT (mm)	AVE CLOT (mm)
None (Control)	21	21	9 x 3	15 x 5	12 x 4
Heparin	24	None			
Dicumarol	12	7 †	5 x 2	15 x 5	10 x 3
Tromexan	12	10 †	1 x 1	14 x 6	7 x 4
Coumadin	10	10	2 x 1	18 x 4	10 x 3

† When no clot formed prothrombin was more than 27 seconds (less than 5%)

In 34 experiments heparin was used No thrombus occurred in 24 when the clotting time was raised at least 1½ times the pre heparin value at the time the electrodes were applied This was true even though frequently the clotting time had returned to its pre heparin value by the end of 1 hour during which time the electric current was applied

In 10 experiments heparin was given and time was allowed for the clotting time to return to the pre heparin value before the current was applied to the vein Thrombosis occurred in 8 of these suggesting that the antithrombotic effect is not significantly prolonged beyond the return of clotting time to normal

Heparin is apparently not consumed in preventing thrombosis In 3 dogs clotting times were obtained at 10 minute intervals after the injection of 0 75 mg /kg of heparin This was repeated the next day with the application of 5 ma of electric current to the external jugular vein for 1 hour A graphic *record of clotting time on both days showed no significant difference (Fig 2)*

The results with dicumarol coumadin and tromexan are also shown in Table 1 Thrombosis always occurred and the clot was comparable in size adherence and consistency with controls when the prothrombin value was between 18 and 5% When the prothrombin value was less than 5% (over 27 seconds) thrombosis did not always occur However excessive bleeding at the neck wound was noted and this made the application of the electrodes difficult

Thrombosis was prevented in only 5 of 12 veins with dicumarol In all instances the prothrombin time was greater than 30 seconds a value of less than 3% Bleeding was excessive on exposure of the vein in the 5 experiments where thrombosis did not occur Thrombi which developed when the prothrombin value was between 18 and 3% ranged from 5 by 2 mm to 15 by 5 mm (average 10 by 8 mm)

In ten coumadin experiments prothrombin times between 15 seconds and 62 seconds (less than 1%) were obtained before the current was applied Thrombosis always developed The smallest clot was 2 by 1 mm in size with a prothrombin time of 62 seconds (less than 1%) The largest was 18 by 4 mm with a prothrombin time of 16 seconds (16%) The average clot size was 10 by 3 mm comparable with our control average

In twelve tromexan experiments prothrombin times between 19 (10%) and 62 seconds were obtained Thrombosis occurred in all but 2 veins In these the prothrombin times were 53 seconds (less than 1%) and 27 1 seconds (5%) and excessive bleeding was noted on exposing the vein The smallest clot was

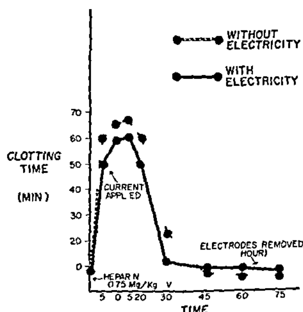


Fig 2 Note that the application of 5 ma of electric current to one external jugular vein did not significantly alter the systemic effect of heparin therefore heparin is not consumed in preventing thrombosis with this method

nly 1 by 1 mm and the largest 11 by 6 mm with an average of 7 by 4 mm A typical clot following the administration of 75 mg of tromexan for 2 days and with a prothrombin time of 30 seconds (less than 5%) is shown in Figure 3 This compares in size and consistency with thrombosis noted in controls[1]

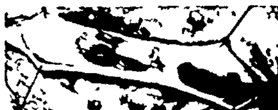


Fig 3 A typical thrombus in the right external jugular vein which formed while the prothrombin time after oral tromexan was 30 seconds (prothrombin value of less than 5%) Bleeding difficult to control

DISCUSSION

These results do not agree with others reported in the literature With this method thrombosis is always prevented by heparin when the clotting time is altered Thrombosis is not prevented by the coumarin derivatives tested except when prothrombin values are below 5% of normal

The mechanism of thrombus formation with this method may be responsible Since thrombosis occurs at the positive electrode electricity may be considered its cause We have been unable to produce thrombosis with small amounts of electricity alone[1] Tissue damage is necessary It occurs mostly under the positive electrode This vessel damage is more likely the cause of thrombosis than is electricity Clinical venous thrombosis also is probably initiated by injury to the vessel wall Localized liberation of tissue thromboplastin produces a clot attached to the area of injury

Quick states that both heparin and coumarin derivatives are supposed to prevent thrombosis but their modes of action are different[8] At what phase of the coagulation mechanism these drugs work is still not clear The coumarin derivatives decrease thrombin by inhibiting the synthesis of prothrombin and accessory factors Heparin inactivates thrombin Much of the confusion about the relative value of anticoagulants is due to unsatisfactory laboratory control

The results in these experiments cast doubt on the clinical value of coumarin derivatives in safe dosage Experimentally low prothrombin values associated with a bleeding tendency must be obtained to prevent thrombosis Small doses of heparin are required Further study seems warranted Clinically thrombosis has already begun when anticoagulants are given We are presently evaluating the extent of propagation of intravascular thrombosis with this method The preventive effect on clot propagation using various anticoagulants will be studied

SUMMARY AND CONCLUSIONS

A method of producing a standard degree of venous thrombosis experimentally utilizing a small electric current has been described Heparin prevents thrombosis when the clotting time is prolonged 1½ or more times the pre heparin value Three coumarin derivatives (tromexan coumadin and dicumarol) do not prevent thromobsis unless the prothrombin value is less than 5% of normal These results suggest the need for further experimental evaluation of these anticoagulants

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THE USE OF DEXTRAN SULFATE AS AN ANTICOAGULANT FOR OPEN HEART SURGERY *

WILLIAM H. WADE AND RAYMOND VICKERS

A search has been made for an anticoagulant to preserve blood for open heart surgery that would be preferable to heparin.[1] While heparin is reasonably satisfactory, it has the great disadvantage that blood so preserved cannot be predictably stored for over 24 hours. In many centers, therefore, including our own, blood is drawn the morning of surgery. It is felt that if one could bank blood satisfactorily for several days prior to surgery, it would be very advantageous.

Experience with dextran sulfate † to date indicates that it is a satisfactory substance for this purpose. This material is an analogue of heparin, being a sulfonated polysaccharide. It is synthesized from glucose and as such is the same substance that is used for a plasma volume expander. It differs from the latter, however, in that molecules of the range of 8 000–10 000 molecular weight are selected and then sulfonated. These processes give the substance its anticoagulant properties.

A considerable experience has now accumulated using this material as an anticoagulant clinically for coronary thrombosis and thromboembolic disease.[2] The present investigation grew out of our experience with this drug as an anticoagulant and concerns its use in preserving blood *in vitro* for transfusion purposes.

METHOD

Anticoagulant Action. Dog's blood was preserved at 4°C with dextran sulfate in different concentrations in siliconized glass bottles and in plastic bags (Fenwal, Ethicon, Inc.). Samples were examined at frequent intervals for clot formation, hemolysis and the state of erythrocyte and platelet preservation.

Survival of Transfused Cells. Canine erythrocytes labeled with radioactive chromium (Cr^{51}) after 4 days storage in dextran sulfate were used as part of an exchange transfusion in 5 dogs. One animal was discarded on the second day because of intercurrent disease. Specimens of blood were taken at 3 day intervals for 15 days from the remaining 4 animals and the radioactivity was measured in a scintillation well type counter. This permitted calculation of the survival times of the transfused cells.

Toxicity and Neutralization. The 5 animals alluded to above which had exchange transfusions with dextranized blood were given hexadimethrine bromide †† in sufficient amount to neutralize the dextran sulfate. The necessary amount of Polybrene was previously calculated from *in vitro* neutralization studies that permitted construction of a graph relating equivalent amounts of dextran sulfate, Polybrene and protamine.[3]

In addition 5 dogs were subjected to a 30 minute cardiopulmonary bypass

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† Dexulate supplied by Glaxo Laboratories Ltd, Greenford, England.

†† [illegible] Laboratories, North Chicago, Ill., and being investigated [illegible] Center by Dr. P. W. Hitchcock.

* [illegible] and Medicine of the University of Missouri Medical [illegible] ool. With the technical assistance of Mr. Robert Stockwood, Dr. James Ozenberger and Dr. Henry McQuade. Supported in part by a grant from the Missouri Heart Association.

with the DeWall pump oxygenator using dextran sulfate as the anticoagulant *for the patient as well as the stored blood* At the conclusion of these experiments the dextran sulfate was neutralized with protamine or polybrene In both groups clotting times were followed at 4 hour intervals

Dextran sulfate was given to human subjects in doses sufficient to provide a concentration of 3 units/ml These individuals were observed for toxic effects

RESULTS

The period for which blood can be preserved free of clots by dextran sulfate is summarized in Figure 1 It has been found that at least 3 units/ml are required to preserve the blood for 4 days

The viability of erythrocytes as measured by the radiochromium survival studies showed a drop in the first 24 hour period similar to that seen with heparin [4] Hematocrit levels, however, did not significantly vary during the postransfusion period

Platelet levels drop to between 50% and 75% of the original in preserved blood during the first 24 hours and then fall off much more slowly A comparison of dextran sulfate, heparin and ACD preserved blood shows the greatest survival (75%) of platelets in the former Plasma hemoglobin determinations on the blood used for cardiopulmonary bypass with the pump oxygenator demonstrated that with dextran sulfate as with heparin the *amount of cell destruction was proportional to the length of the perfusion* and was of the same order

The osmotic fragility of the blood remained normal throughout the perfusion as measured by the immediate and the 24 hour incubation methods

We failed to find a case of tachycardia hypotension or other objective findings of toxicity in 12 dogs and 9 of 12 humans who received the drug The remaining 3 subjects reported nausea during its rapid intravenous injection

Some difficulty was encountered in the calculation of the correct amount of protamine sulfate required for neutralization The achievement of a perfectly normal clotting time was, however, found to be unnecessary to produce satisfactory hemostasis Because of the long acting nature of this new anticoagulant, the patients clotting times had to be followed for as long as 12 hours after operation but no other special difference was encountered

DISCUSSION

The use of heparin as an anticoagulant for stored blood is common for open heart surgery but is fraught with the major disadvantage that it is effective for

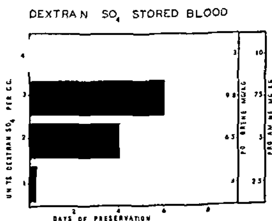


Fig 1 The length of satisfactory preservation of dogs blood with dextran sulfate

only about 24 hours Our study of dextran sulfate has been directed toward extending this period *In vitro* studies have clearly shown that dextran sulfate is an effective anticoagulant for at least a 4 day period. Furthermore, preliminary studies in dogs and humans indicate that in the doses required it is nontoxic. Studies to date indicate that there is no *in vitro* evidence of damage to erythrocytes as indicated by the normal osmotic fragility and by the lack of abnormal hemolysis following circulation through the DeWall pump oxygenator. *In vivo* survival studies on erythrocytes by the radioactive chromium method suggest fairly rapid loss of approximately 50% of the cells within 24 hours after storage for 4 days in dextran sulfate but this is at variance with the fact that there are no significant hematocrit changes in the animals during this period. Work is in progress to further elucidate this situation. At any rate blood preserved in heparin for only 24 hours shows a similar early destruction of erythrocytes and this is of little clinical significance. It has been clearly shown that the dextran sulfate can be as readily neutralized by protamine or Polybrene as heparin. A predictable relationship exists between the amount of dextran sulfate used and the amount of protamine or Polybrene necessary for neutralization. It is our feeling that dextran sulfate is a satisfactory anticoagulant for preserving blood for open heart surgery and has the advantage over heparin that blood so preserved can be banked for several days prior to the operation.

SUMMARY

Studies on the use of a new anticoagulant, dextran sulfate, for preserving blood for open heart surgery are presented The data suggest that this material is a satisfactory anticoagulant for this purpose and that blood may be stored satisfactorily for several days prior to surgery.

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Addendum Recent studies of Cr^{51} tagged erythrocytes stored in dextran sulfate indicate that the apparent destruction of 50% in the first 24 hour period is artefactual and is due to inadequate labelling of the red cells One clinical case has undergone open heart surgery using dextran sulfate as the anticoagulant with success.

THE ANTIHEPARIN PROPERTIES OF THE ANTIHISTAMINES TRANQUILIZERS AND CERTAIN ANTIBIOTICS *

RUSSELL M NELSON C GORDON FRANK AND JAMES O MASON

Compounds known to reverse the anticoagulant properties of heparin are protamine sulfate toluidine blue[1] and recently polybrene[2] Apparently the mechanism for this antagonism is in the formation of stable precipitates from these basic drugs reacting with the acidic heparin molecule

Our interest in this problem developed during the course of experimentation with drugs possibly capable of inducing temporary cardiac arrest during extracorporeal circulation There we noted that the addition of diphenhydramine (Benadryl) to heparinized blood initiated coagulation

Therefore further studies were planned to determine possible antiheparin effects of other antihistamines and subsequently the tranquilizers and antibiotics as well

METHOD

Three methods of evaluation were employed 1) the *in vitro* mixing of individual drugs with heparin 2) the determination of the ratio of drugs to heparin required to cause coagulation of previously heparinized blood 3) the determination of possible effects of the drugs on the coagulation time of otherwise normal dogs

The drugs employed in this study were as follows

Antihistamines chlorprophenpyridamine maleate (Chlortrimeton) promethazine hydrochloride (Phenergan) cyclizine lactate (Marezine) diphenhydramine hydrochloride (Benadryl) tripelennamine hydrochloride (Pyribenzamine)

Tranquilizers promazine hydrochloride (Sparine) chlorpromazine hydrochloride (Thorazine) hydroxyzine hydrochloride (Atarax) prochlorperazine (Compazine) perphenazine (Trilafon)

Antibiotics kanamycin sulfate (Kantrex) erythromycin glucoheptonate (Ilotycin) tetracycline hydrochloride (Achromycin) chlortetracycline hydrochloride (Aureomycin) penicillin G potassium chloramphenicol (Chloromycetin) dihydrostreptomycin

Miscellaneous Drugs protamine sulfate hexadimethrine bromide (Polybrene) heparin (Liquaemin sodium—Organon—10 mg /ml) procaine amide hydrochloride (Pronestyl) phenylephrine hydrochloride (Neo synephrine) dimethylamino trimethoxy triphenyl propene HCl (WIN 5191)

For the sake of brevity further reference to these agents will be by the trade name in those instances where that name is generally used

In Vitro Reactions Between Aqueous Heparin and Solutions of the Drugs Each of the drugs was added to separate test tubes containing 10 mg aqueous heparin and the subsequent reactions were observed The amount of test drug added to the heparin is indicated in the table under results

Dose Ratio of Drug to Heparin Necessary to Coagulate Heparinized Dog Blood Arterial blood was obtained from dogs anesthetized with open drop

* From the Department of Surgery University of Utah College of Medicine Supported by grants from U S Public Health Service (H 30 0) Utah Heart Association and the American Heart Association

ether and collected in a graduated Erlenmeyer flask containing the amount of heparin necessary to make the final concentration of heparin 1 mg /100 ml of blood Ten milliliter aliquots of this blood (each containing 1 mg of heparin) were then transferred to test tubes The drug to be tested was added to the heparinized blood in graded doses to determine the amount necessary for coagulation

Coagulation Time Studies in Dogs Three dogs were utilized for this study The first dog was injected intravenously with 7 2 mg of Phenergan (3 7 mg /kg) The second dog was injected intravenously with 8 9 mg of Thorazine (3 5 mg /kg) The third dog serving as a control, was injected intravenously with 2 ml of 9% saline solution The dosages of these drugs were approximately that advised in clinical usage The Lee White method was employed to determine the clotting times First the normal clotting times were determined and then the clotting times were serially determined after the administration of the two drugs and the saline

Table 1 In Vitro Reactions Between Aqueous Heparin and Solutions of Test Drugs

DRUG	DRUG ADDED	HEPARIN ADDED	RESULTS
Antihistamines			
Chlortrimeton	10 mg	10 mg	white precipitate
Phenergan		"	"
Marezine			"
Benadryl		"	
Pyribenzamine		"	" "
Tranquilizers			
Sparine	5 mg	10 mg	white precipitate
Thorazine		"	"
Atarax	"	"	" "
Compazine			" "
Trilafon		"	"
Antibiotics			
Kanamycin	10 mg	10 mg	white precipitate
Erythromycin			
Achromycin			yellow precipitate
Aureomycin		"	no reaction observed
Chloromycetin		"	white precipitate
Dihydrostreptomycin		"	"
Penicillin	30 000 units		no reaction observed
Miscellaneous			
Protamine sulfate	10 mg	10 mg	white precipitate
Polybrene		"	" "
Pronestyl			no reaction observed
Neo synephrine			
Saline		"	
WIN 5494		"	white precipitate

RESULTS

In Vitro Reactions Between Aqueous Heparin and Solutions of the Drugs The data show that all the antihistaminic and tranquilizer drugs tested reacted with heparin to form a precipitate All of the antibiotic drugs tested with the exception of penicillin and aureomycin similarly caused precipitation when added to heparin These results are listed in Table 1 Similar precipitation was observed *in vitro* when higher or lower concentrations of these drugs were used

Dose Ratio of Drug to Heparin Necessary to Coagulate Heparinized Dog Blood These data show that all the antihistamines and tranquilizers tested caused coagulation of heparinized dog blood provided large enough doses were employed Table 2 summarizes these results and indicates the relatively

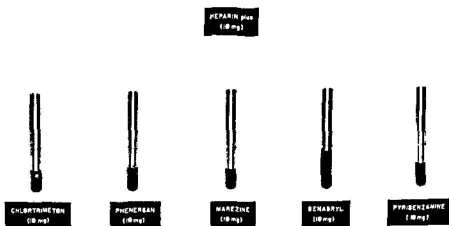


Fig 1 Reactions obtained when solutions of antihistamines are added to aqueous heparin

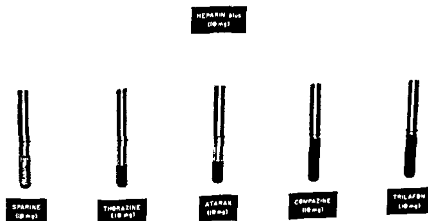


Fig 2 Reactions obtained when solutions of tranquilizers are added to aqueous heparin

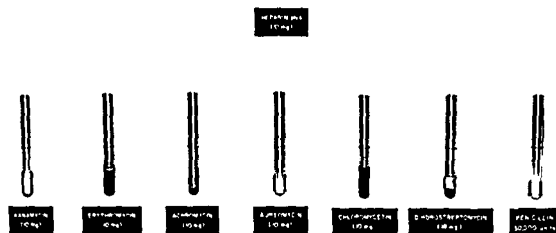


Fig 3 Reactions obtained when solutions of antibiotics are added to aqueous heparin

large doses required in most instances to effect coagulation Hemolysis was noted in all reactions Protamine sulfate in the usual dose ratio of 1 mg /1 mg heparin caused coagulation

Table 2 Dose Ratio of Drug to Heparin Necessary to Coagulate Heparinized Dog Blood

DRUG	MINIMUM AMOUNT OF DRUG NECESSARY FOR COAGULATION OF BLOOD CONTAINING HEPARIN (4 mg /10 ml)	APPROXIMATE COAGULATION DOSE RATIO OF DRUG TO HEPARIN (mg /mg)	HEMOLYSIS
Antihistamines			
Chlortrimeton	200 mg	500/1	+
Phenergan	15 mg	38/1	+
Marezine	25 mg	63/1	+
Benadryl	50 mg	125/1	+
Pyribenzamine	10 mg	25/1	+
Tranquilizers			
Sparine	25 mg	63/1	+
Thorazine	5 mg	13/1	+
Atarax	15 mg	38/1	+
Compazine	10 mg	25/1	+
Trilafon	5 mg	13/1	+

Coagulation Time Studies In Dogs The determination of the coagulation time by the Lee White method in each of the 3 dogs tested was initially 15 minutes (±1 min) Subsequent determinations up to 2 hours after the administration of Phenergan (37 mg /kg) Thorazine (35 mg /kg) and saline to each respective experimental animal all were in the range of 13 to 17 minutes Thus in the dosages tested no demonstrable effect on normal blood coagulation time was observed

DISCUSSION

Fischer and Astrup[3] showed in 1935 that the neutralization of heparin by basic compounds is due to a reaction similar to that of a weak acid reacting with a weak base They explained that the salt resulting from this reaction dissociated according to the law of mass action This is possibly the mechanism of the neutralization of heparin by the drugs employed in this study If this is the case, there are undoubtedly many other compounds with free amine groups or otherwise basic character that could be expected to exhibit a similar reaction With the exception of an article by Waisbren and Glick[4] in 1950 that the addition of aureomycin to human plasma *in vitro* produces a significant decrease in the heparin concentration, it is surprising that incompatibilities of such commonly used drugs have not been previously reported Further work in our laboratory is under way to study these reactions quantitatively

We have also found that very high, nonphysiologic doses of some of the antihistaminic and tranquilizer drugs cause a general precipitation of a large amount of human serum proteins Analysis in the Tiselius apparatus shows that this is not specific for any particular protein fraction

From these observations, it is evident that certain practical implications may be derived from this demonstration of the antiheparin properties of these groups of drugs First, the amount of effective dose of each drug *in vivo* is reduced during concomitant administration of these incompatible agents Second, in order to avoid the formation of precipitates in fluids prepared for intravenous administration to patients, mixtures of these incompatible drugs should be avoided Third the increasingly common practice of adding antihistamines to donor blood to prevent nonhemolytic transfusion reactions is contra indicated in patients who are to be heparinized, such as those undergoing open heart surgery employing extracorporeal circulation

SUMMARY

The antiheparin properties of the antihistamines, tranquilizers and certain antibiotics have been demonstrated A precipitate formed when aqueous solutions of most of the drugs used in this experiment were added to aqueous heparin Large doses of these drugs were shown to inactivate heparin and allow heparinized blood to coagulate No apparent alteration of normal coagulation time in dogs subsequent to the intravenous administration of these drugs was observed The importance of avoiding these drug incompatibilities in the clinical situation has been stressed

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Extracorporeal Circulation

TISSUE OXYGEN "TENSION" AT VARIOUS FLOW RATES OF EXTRACORPOREAL CIRCULATION*

SEYMOUR I. SCHWARTZ, JAMES A. DEWEESE, FAUSTINO N. NIGUIDULA, PAUL V. GABLE, AND EARLE B. MAHONEY

The effect of various rates of extracorporeal flow upon the physiologic function of critical organs is of great importance Since metabolic activity requires available oxygen, the measurement of the tissue oxygen "tension" reflects the adequacy of extracorporeal flow The use of polarography and the application of stationary platinum electrodes in the measurement of oxygen concentration in the tissue, affords a method of determining the oxygen availability in critical organs prior to and during the establishment of cardiac bypass

METHOD

Fine platinum electrodes were inserted into the brain, liver, kidney, and leg muscle of pentothal anesthesized dogs under direct vision The reference calomel electrode was placed in the subcutaneous tissue of the neck. Polyethylene catheters were threaded into the aorta and vena cava *via* the femoral vessels for measurements of pressures Respirations were controlled by means of an automatic positive pressure compressed air respirator, and a standard right thoracotomy was then performed The superior vena cava, inferior vena cava, and left subclavian artery were then cannulated

The electrodes were connected to a polarograph and control readings were recorded after stabilization had occurred A combination of the DeWall bubble oxygenator and sigmamotor pump was used to provide an extracorporeal circulation Four groups of dogs, each containing 1 to 5 animals, were studied using flow rates of 100, 80, 60, and 10 cc /kg /min The flow of

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means of the pump oxygenator Arterial and venous pressures were simultaneously recorded during this period, pH, lactic acid, sodium, potassium, CO_2, and arteriovenous oxygen differences were also determined.

RESULTS

Brain tissue oxygen tensions (Figure 1a), as recorded in microampere current flow, rose appreciably above the control level at flows of 100, 80, and 60 cc /kg /min At 10 cc /kg /min flow, however, the oxygen tension was maintained at the control level and did not rise Tissue oxygen tensions of the kidney (Figure 1b) rose above the control value of flows of 100 cc /kg /min , and were maintained at control level at 80 cc /kg /min. There was a small decrease below the control value during flows of 60 and 10 cc /kg /min

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Skeletal muscle (Figure 1c) demonstrated oxygen tensions at or above the control with flows of 100 80 and 60 cc /kg /min but when a rate of 40 cc /kg /min was used *the tension fell below control value* The changes in the tissue oxygen tension of the liver (Figure 1d) were erratic and failed to reveal any statistically significant pattern

Regrouping *the results* according to rate of flow demonstrated that at 100 cc /kg /min (Figure 2a) and 80 cc /kg /min (Figure 2b) the tissue oxygen *tension in all the organs studied was maintained above the* control level At 60 cc /kg /min (Figure 2c) the brain and skeletal muscle maintained oxygen tension above control but the kidney oxygen tension fell slightly below the control level At 40 cc /kg /min (Figure 2d) there was a distinct dropoff below the values obtained during the control period for the muscle and kidney and the tissue oxygen tension in the brain fell off and reached the control level

The alterations in tissue oxygen tension paralleled changes in arterial pressure and in arteriovenous oxygen differences At 100 cc /kg /min flow *arterial pressure was maintained well above the prepumping control value* At 80 cc and 60 cc /kg /min flow arterial pressure was maintained at approximately the prepump control level However at 40 cc /kg /min flow *there was a significant decrease in arterial pressure The A V oxygen difference* decreased over the one hour period of extracorporeal circulation at the flow rates of 100 80 and 60 cc /kg /min However at flow rates of 40 cc /kg /min there was a slight increase in A V oxygen difference The changes *in tissue* oxygen tension could not be related to changes in blood pH lactic acid CO or electrolytes to any significant degree

DISCUSSION

The presently available pump oxygenators readily provide a high flow of 100 cc /kg /min in children and small adults However in most adults a low flow must be relied upon It is therefore of practical import to determine *the effects* of reduced circulation on *critical organs Metabolic studies* demonstrated no difference in oxygen saturation or pH or CO_2 with flows varying between 32 and 60 cc /kg /min [1] It has been shown that progression of acidosis during total body perfusion was accentuated by perfusion rates below 40 cc /kg /min [2] Studies of liver function during extracorporeal circulation with low flow rates varying between 29 and 63 cc /kg /min indicated that hepatic metabolism was maintained at normal and oxygen availability was not markedly depressed despite a slight decrease in hepatic blood flow [3] Our studies indicate that maintenance of a blood flow of 60 cc /kg /min *maintains oxygen tension* at control levels in the brain and striated muscle and at slighty below control levels in the kidney under the conditions of these experiments At 40 cc /kg /min the tissue oxygen tension in liver kidney *and muscle are all depressed* but the brain is maintained at normal

These findings are consistent with metabolic studies in which it was felt that a flow rate below 40 cc /kg /min was critical for changes of acidosis [2] *Polarographic measurements* of oxygen tension in whole blood showed a rise from 50 mm up to a level of 700 mm in the oxygenator with the usually employed flow rates [4] It is therefore apparent that there is a sufficient super*saturation of blood in the oxygenator* to compensate for a lower flow rate and thus maintain tissue oxygen tension at the control level

Fig 1. Relationship between tissue oxygen "tension", measured in microampere current flow, and extracorporeal flow rate in 1a brain, 1b kidney, 1c skeletal muscle and 1d liver.

Fig 2 Changes in tissue oxygen "tension of brain, kidney, skeletal muscle, and liver at extracorporeal flow rates of 2a 100 cc/kg/min, 2b 80 cc./kg/min, 2c 60 cc./kg/min, and 2d 40 cc./kg/min

Skeletal muscle (Figure 1c) demonstrated oxygen tensions at or above the control with flows of 100, 80, and 60 cc./kg./min., but when a rate of 40 cc./kg./min. was used, the tension fell below control value. The changes in the tissue oxygen tension of the liver (Figure 1d) were erratic and failed to reveal any statistically significant pattern.

Regrouping the results according to rate of flow demonstrated that at 100 cc./kg./min. (Figure 2a) and 80 cc./kg./min. (Figure 2b), the tissue oxygen tension in all the organs studied was maintained above the control level. At 60 cc./kg./min., (Figure 2c) the brain and skeletal muscle maintained oxygen tension above control; but the kidney oxygen tension fell slightly below the control level. At 40 cc./kg./min. (Figure 2d), there was a distinct dropoff below the values obtained during the control period for the muscle and kidney, and the tissue oxygen tension in the brain fell off and reached the control level.

The alterations in tissue oxygen tension paralleled changes in arterial pressure and in arteriovenous oxygen differences. At 100 cc./kg./min. flow, arterial pressure was maintained well above the prepumping control value. At 80 cc and 60 cc./kg./min. flow, arterial pressure was maintained at approximately the prepump control level. However, at 40 cc./kg /min. flow, there was a significant decrease in arterial pressure. The A-V oxygen difference decreased over the one hour period of extracorporeal circulation at the flow rates of 100, 80, and 60 cc./kg./min. However, at flow rates of 40 cc./kg./min., there was a slight increase in A-V oxygen difference. The changes in tissue oxygen tension could not be related to changes in blood pH, lactic acid, CO_2, or electrolytes to any significant degree.

DISCUSSION

The presently available pump oxygenators readily provide a "high-flow" of 100 cc./kg./min. in children and small adults. However, in most adults, a "low-flow" must be relied upon. It is, therefore, of practical import to determine the effects of reduced circulation on critical organs. Metabolic studies demonstrated no difference in oxygen saturation, or pH, or CO_2 with flows varying between 32 and 60 cc./kg./min.[1] It has been shown that progression of acidosis during total body perfusion was accentuated by perfusion rates below 40 cc./kg./min.[2] Studies of liver function during extracorporeal circulation with low flow rates varying between 29 and 63 cc./kg./min. indicated that hepatic metabolism was maintained at normal, and oxygen availability was not markedly depressed, despite a slight decrease in hepatic blood flow.[3] Our studies indicate that maintenance of a blood flow of 60 cc./kg./min. maintains oxygen tension at control levels in the brain and striated muscle, and at slighty below control levels in the kidney under the conditions of these experiments. At 40 cc./kg./min., the tissue oxygen tension in liver, kidney, and muscle are all depressed, but the brain is maintained at normal

These findings are consistent with metabolic studies in which it was felt that a flow rate below 40 cc./kg./min. was critical for changes of acidosis.[2] Polarographic measurements of oxygen tension in whole blood showed a rise from 50 mm. up to a level of 700 mm. in the oxygenator with the usually employed flow rates.[4] It is, therefore, apparent that there is a sufficient supersaturation of blood in the oxygenator to compensate for a lower flow rate and thus maintain tissue oxygen tension at the control level.

1 a

1 b

1 c

1 d

Fig 1 Relationship between tissue oxygen "tension" measured in microampere current flow and extracorporeal flow rate in 1a brain 1b kidney 1c skeletal muscle and 1d liver

2 a

2 b

2 c

2 d

Fig 2 Changes in tissue oxygen "tension" of brain, kidney, skeletal muscle and liver at extracorporeal flow rates of 2a 100 cc/kg/min, 2b 80 cc/kg/min, 2c 60 cc/kg/min, and 2d 40 cc/kg/min

SUMMARY

The use of pump oxygenators for maintaining an extracorporeal circulation has created a need for the determination of the minimal but safe blood flow rate necessary to maintain homeostasis. Oxygen tensions of various tissues including the brain, kidney, liver, and skeletal muscle have been measured with a polarograph and a stationary platinum electrode during a one hour period of total body perfusion with a bubble oxygenator at blood flow rates of 40, 60, 80, and 100 cc./kg./min. Under the conditions of these experiments a flow rate of 60 cc./kg./min. seems necessary to maintain O_2 concentration at or above control levels in these critical areas.

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STUDIES OF ORGAN FLOW AND METABOLISM DURING EXTRACORPOREAL CIRCULATION *

KEITH REEMTSMA, M. MARTIN HALLEY, WILLIAM H. LETSON, WHITE E. GIBSON III, PATRICK HANLEY III, AND OSCAR CREECH, JR.

The prolonged use of extracorporeal circulation in humans is frequently accompanied by serious complications including various neurologic disturbances suggestive of cerebral damage. A study of cerebral blood flow and metabolism during extracorporeal circulation was reported last year.[2] The scope of the study has been extended and the present report deals with blood flow and oxygen consumption in the brain, kidney, intestine, and extremity during extracorporeal circulation.

METHOD

A total of 40 mongrel dogs, ranging in weight from 9 to 15 kg., was used. Anesthesia was induced with nembutal administered intravenously in dosages of 30 mg./kg. The preparation for the study of cerebral blood flow has been reported previously in detail.[3] For the measurement of renal blood flow, one renal vein was cannulated through the opposite renal vein and this catheter was connected to a Y tube. Another catheter in the femoral vein was connected to the Y tube and the circuit completed. The third limb of the Y tube

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was used to measure flow. Blood was collected in a calibrated burette over a measured period of time, and the organ flow in ml./100 gm. of tissue was obtained by appropriate calculations. Similar procedures using the venous outflow principle were used to study flow of the intestine and extremity by cannulations of the mesenteric vein and femoral vein respectively.

Extracorporeal circulation was performed using a bubble oxygenator. Continuous arterial blood pressure was measured by a strain gauge manometer and recorded continuously. Rectus muscle, organ, and perfusate temperatures were monitored by means of a telethermometer, except in the cerebral preparation. Oxygen content was measured by the technique of Scholander and Roughton.[4]

RESULTS

Analyses of blood flow determinations demonstrated differences in the responses of various organs to extracorporeal circulation. Cerebral blood flow remained relatively constant except at low perfusion rates and was closely correlated with arterial pressure. Renal blood flow fell during extracorporeal circulation and was not closely correlated with arterial pressure. Mesenteric and femoral flow increased during extracorporeal circulation and these changes were not closely correlated with arterial pressure.

The pattern of oxygen consumption varied in different organs in response to extracorporeal circulation. Cerebral oxygen consumption fell abruptly with the onset of extracorporeal circulation. The arteriovenous oxygen difference decreased, with a marked rise in venous oxygen. However, renal oxygen consumption rose slightly during extracorporeal circulation with an increase in the arteriovenous oxygen difference and a decrease in the venous oxygen content. The mesenteric preparations showed a pattern of response similar to that in the cerebral study, with a decrease in oxygen consumption and an increase in venous oxygen accompanying the onset of extracorporeal circulation. The extremity preparations demonstrated relatively constant oxygen consumption with marked increases in flow during extracorporeal circulation. As *in the cerebral and mesenteric preparations, a marked rise in venous* oxygen was noted in the extremity. Temperatures were maintained within 2°C. of control values.

DISCUSSION

The interpretation of organ flow involves the consideration of both pressure and resistance. This study confirms that cerebral flow is determined mainly by blood pressure rather than by cerebral vascular resistance. However, in the kidney, intestine, and extremity, vascular resistance is an important determinant of flow. This factor explains the lack of close correlation between pressure and flow in these areas.

The pattern of response in oxygen consumption varied considerably among the different groups. With the onset of extracorporeal circulation, oxygen consumption increased slightly in the kidney and extremity. Renal flow decreased significantly as has been reported previously by Beall *et al.*[1] The slight increase in renal oxygen consumption was accompanied by a marked increase in the arteriovenous oxygen difference. By contrast, extremity flow increased markedly, and the slight increase in oxygen consumption was accompanied by a marked rise in venous oxygen and a decrease in the arteriovenous oxygen difference.

Unlike the kidney and extremity, the brain and intestine demonstrated a decrease in oxygen consumption with the onset of extracorporeal circulation The phenomenon of decreased oxygen consumption in the brain has been

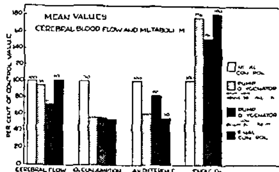


Fig 1 Graph showing the mean values in 19 experiments

and venous oxygen content

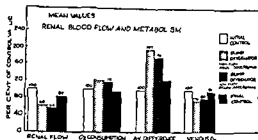


Fig 2 Graph showing the mean values in 9 experiments expressed as percentage of control levels of renal flow, oxygen consumption arteriovenous oxygen difference, and venous oxygen content

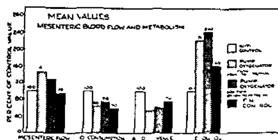


Fig 3 Graph showing the mean values in 9 experiments expressed as percentage of control levels, of mesenteric flow, oxygen consumption, arteriovenous oxygen difference, and venous oxygen content

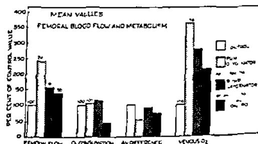


Fig 4 Graph showing the mean values in 9 experiments, expressed as percentage of control levels, of femoral flow oxygen consumption arteriovenous oxygen difference and venous oxygen content

discussed in detail [3] and various possible explanations presented The present study demonstrates the same response in the intestine With the temperature relatively constant the fall in oxygen consumption occurred despite significant increase in flow

SUMMARY

1 Cerebral blood flow during extracorporeal circulation is determined mainly by arterial pressure Renal, mesenteric, and femoral flow vary with resistance and are not closely correlated with pressure

2 Oxygen consumption in the kidney and extremity *increased slightly with* extracorporeal circulation The intestine and brain demonstrated decreased oxygen consumption despite adequate flow

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A STUDY OF CHANGES IN PERIPHERAL FLOW AND RESISTANCE AS ASSOCIATED WITH TOTAL BODY PERFUSION *

JAY L. ANKENEY, PETER H. VILES, AND JACK R. LEONARDS

Hypotension often follows total cardiopulmonary bypass The cause for this shocklike state after extracorporeal perfusion is unclear but is probably related to changes in flow and peripheral vascular resistance Therefore, in an attempt to explain this sequela of perfusion, the following experiments were performed in which pressure and flow were measured and resistance calculated in dogs before, during, and following total body perfusion These experiments also made possible a study of flow and resistance with changes in pump perfusion rate, increased venous pressure, and variation in pCO_2

METHOD

Mongrel dogs were anesthesized with Surital, 12 5 mg./kg After intubation, the right chest was entered through the fifth intercostal space and ventilation maintained with an intermittent positive pressure respirator using room air Vena caval catheters were placed and a cannula inserted into the right femoral artery. During perfusion, blood from the cavae drained by gravity into a venous reservoir, and after passing through a rotating disc oxygenator, was pumped through the arterial cannula by an occlusive roller type pump which delivers a constant stroke volume even against markedly elevated inflow resistance.

In 6 experiments, blood flow through the left femoral artery was measured by a Gregg type rotameter.[1] In 2 other animals, a rotameter was inserted into the left brachial artery with the left vertebral artery ligated In this way, all of the blood flowing to the left upper extremity was monitored Even though flow through collateral channels was not measured in the femoral artery series, comparison of results from both sets of experiments gave similar findings Mean arterial pressure was measured from the afferent limb of the rotameter and venous pressure recorded in the inferior vena cava during femoral flow studies and in the superior vena cava during brachial artery flow experiments The rotameter was calibrated at the end of each experiment using blood from the perfused dogs, and the venous and arterial pressure transducers calibrated at the beginning and end of each experiment The rotameter flow, arterial and venous pressures, and EKG were recorded photographically by a multiple channel oscilloscope

In all experiments, flow, arterial and venous pressure were measured under control conditions, during extracorporeal bypass and following perfusion Peripheral flow and pressure were changed before and after perfusion either by bleeding or the administration of whole blood During perfusion, the arterial perfusion rate was kept constant at 75 cc /kg /min except during short intervals when the perfusion rate was increased or decreased The duration of perfusion varied from 60 to 90 minutes One hundred per cent oxygen

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* From the Department of Surgery, Western Reserve University, School of Medicine, and University Hospitals of Cleveland Cleveland, Ohio Supported by a research grant from the Cleveland Area Heart Society with the technical assistance of Miss Lorna Cox

was passed through the oxygenator except when a mixture of 5% CO_2 and 95% O_2 was used to raise pCO_2

Since, during perfusion, blood loss into the chest was returned to the venous reservoir by means of a suction pump, the amount of blood pooled in an animal could be calculated by comparing the blood volume in the extracorporeal circuit before and after bypass.

Resistance (R) was calculated according to the equation $R = P/F$, where P represents mean arterial pressure in mm. of Hg and F is mean flow in cc./min. Since a linear relationship between pressure and flow does not obtain,[2] the R factor was calculated for purposes of comparison at similar flow rates during control, perfusion, and postperfusion periods

RESULTS

Effect of perfusion upon peripheral flow and resistance. As shown in Table 1, all but 1 animal (137) had mean arterial blood pressures below control levels immediately following perfusion In 6 of the 7 hypotensive dogs, peripheral flow was also reduced below control values Animal 152 showed hypotension despite a higher peripheral flow. Consideration of resistance as described below made possible the determination of the primary basis for hypotension as indicated.

Table 1 Peripheral Flow and Pressure Before and After Perfusion

EXPERIMENT	PERIPHERAL FLOW (cc/min)	PRESSURE (mm Hg)	BASIS FOR HYPOTENSION
137 *Femoral*			
Control	40	80	Stable
Postperfusion	41	84	
141 *Femoral*			
Control	44	102	Decreased
Postperfusion	32	60	Resistance
152 *Femoral*			
Control	32	92	Decreased
Postperfusion	38	84	Resistance
153 *Femoral*			
Control	45	120	Decreased
Postperfusion	28	80	Resistance
154 *Femoral*			
Control	26	100	Decreased
Postperfusion	24	53	Resistance
156 *Femoral*			
Control	27	120	Decreased
Postperfusion	15	85	Flow
168 *Brachial*			
Control	38	70	Decreased
Postperfusion	28	23	Resistance
169 *Brachial*			
Control	52	100	Decreased
Postperfusion	30	64	Flow

Comparison of pre and postperfusion blood pressure at similar flow rates (Table 2), indicates that 4 animals (141, 152, 154 and 168) remained hypotensive with peripheral flow equal to control values Therefore, these 4 dogs showed a marked decrease in peripheral vascular resistance which was the primary cause for the postperfusion hypotension These 4 dogs are referred to as the low resistance group In two instances (156 and 169), peripheral vascular resistance remained unchanged following perfusion, indicating that decreased flow was the primary cause of the immediate postperfusion hypotension (low flow group)

Table 2. Pressure at Similar Peripheral Flow

EXPERIMENT	PERIPHERAL FLOW (cc/min)	PRESSURE (mm Hg)	RESISTANCE R=P/F
137			
Control	40	80	2.0
Perfusion	36	78	2.2
Postperfusion	42	88	2.1
141			
Control	44	102	2.3
Perfusion	45	73	1.7
Postperfusion	43	67	1.6
152			
Control	32	92	2.9
Perfusion	30	82	2.8
Postperfusion	38	81	2.4
154			
Control	21	90	4.3
Perfusion	24	63	2.6
Postperfusion	22	52	2.3
156			
Control	20	97	4.9
Perfusion	18	84	4.6
Postperfusion	19	95	5.0
168			
Control	52	76	1.5
Perfusion	51	84	1.6
Postperfusion	52	60	1.1
169			
Control	52	100	1.9
Perfusion	58	89	1.5
Postperfusion	48	87	1.8

The response of peripheral flow and resistance to the infusion of whole blood in representative animals from each of these two groups is shown in

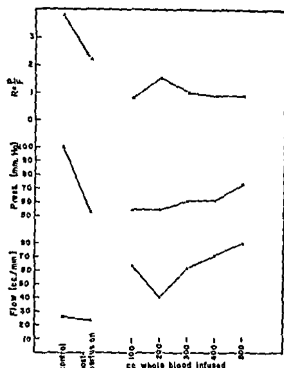


Fig 1 Postperfusion hypotension primarily due to decreased peripheral resistance Response to infusion of whole blood Experiment #154

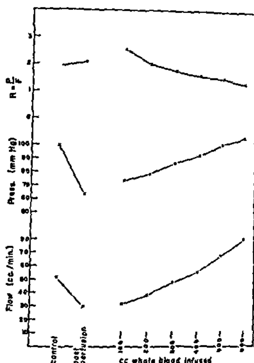


Fig 2 Postperfusion hypotension primarily due to decreased flow Response to infusion of whole blood Experiment #169

Figures 1 and 2 Figure 1 shows that animal 154 (*low resistance group*) remained hypotensive following infusion of 500 cc of whole blood despite a fourfold increase in peripheral flow On the other hand, animal 169 (low flow group) responded to the infusion of 500 cc of whole blood by returning to near control flow and resistance values

Table 2 also shows that resistance fell during perfusion in 4 experiments (141, 154, 156 and 169) and remained at control levels in 3 others There was no correlation, however, between changes in resistance during perfusion and the state of the vascular bed in the postperfusion period

Effect of changing perfusion flow rate upon peripheral flow and resistance Table 3 and Figure 3 show that increasing perfusion flow rate from 75 cc /kg /min to 150 cc /kg /min resulted in a progressive fall in peripheral vascular resistance At flow rates greater than 100 cc /kg /min , there was a marked fall in resistance and in three instances a fall in blood pressure as well

Effect of increasing venous pressure upon peripheral flow. Figure 4 depicts 4 experiments in which venous pressure was progressively increased At venous pressures greater than 20 mm Hg flow to the periphery decreased

Effect of increased pCO_2 upon peripheral flow and resistance. In 5 experiments, arterial pCO_2 during perfusion was increased from 25 to 60 mm Hg Three animals showed no change in peripheral flow or resistance, one showed slight increase and one a slight decrease in these values

DISCUSSION

The cause of decreased peripheral resistance following perfusion is not clear However in the low resistance group of dogs, the plasma hemoglobin

Table 3 Effect of Changing Perfusion Rate Upon Peripheral Flow and Resistance

EXPERIMENT	PERFUSION RATE (cc/kg/min)	PERIPHERAL FLOW (cc/min)	PRESSURE (mm Hg)	RESISTANCE R=P/F
141	75	27	56	2 1
	90	30	68	2 3
	130	45	73	1 6
152	70	21	77	3 2
	105	31	101	3 0
	115	39	114	2 9
	145	45	107	2 4
153	70	21	82	3 9
	95	26	85	3.3
	110	38	92	2 4
	140	38	97	2.5
154	75	20	58	2 9
	100	28	76	2 7
	130	30	82	2 8
	150	37	57	1 6
156	75	13	60	4 7
	90	23	104	4.5
	150	59	106	1 9
168	75	65	71	1 1
	100	76	97	1 3
	120	100	88	0 8
169	75	86	100	1.2
	100	116	107	0 9
	130	158	121	0 7

was elevated above 250 mg /100 cc In several of these same animals, however, this degree of hemoglobinemia did not produce significantly decreased resistance during perfusion Therefore, hemoglobinemia is probably not responsible for the dilated postperfusion peripheral vascular bed

The decreased peripheral flow that occurred in 6 of 8 dogs implies decreased cardiac output or shunting of blood from the periphery into other channels such as the splanchnic bed Work to be published from this laboratory indicates that the low flow is due primarily to a decreased cardiac output which probably is a consequence of a decreased effective circulating blood volume due to sequestration of blood into vascular pools

Clinically, we have observed patients after extracorporeal bypass with hypotension due primarily to either low output or decreased resistance In the one group, blood pressures returned to normal levels after the administration of several hundred cubic centimeters of blood, whereas in the second group, administration of blood failed to raise the pressure but vasopressors resulted in a favorable response

Clinically, we have also observed that systemic pressure during perfusion remains lower than anticipated for what is considered to be a high perfusion flow rate Therefore, it was interesting to note that increasing the perfusion rate experimentally resulted in a decrease in peripheral vascular

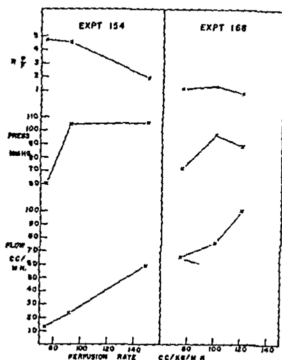


Fig 3 The effect of changing perfusion rate upon peripheral flow and resistance

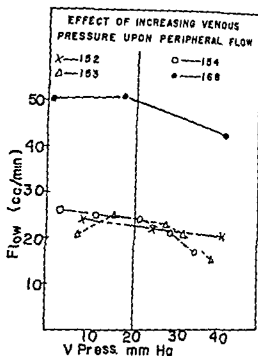


Fig 4

resistance The rise in peripheral flow did not parallel the increase in perfusion rate This implies that at high flow rates blood is not circulated to the periphery but goes to other systems such as the splanchnic region Consistent with these observations is the fact that increasing the pump flow rate resulted in pooling of blood by the animal

The observation that increasing venous pressure above 20 mm of Hg results in decreased flow has clinical importance During perfusion, venous pressure should be monitored and every effort made to keep it below 20 mm of Hg This is especially true for the superior vena cava as cerebral blood flow is likewise affected by elevated pressure

SUMMARY

1 In 7 out of 8 dogs, hypotension followed extracorporeal bypass

2 Postperfusion hypotension is due to two factors 1) decreased flow and/or 2) decreased peripheral resistance

3 During cardiopulmonary bypass, peripheral resistance decreased in 4 of 7 dogs

4 Increasing pump perfusion rate resulted in decreased peripheral vascular resistance

5 Peripheral blood flow falls if the venous pressure rises above 20 mm of Hg

6 Changing arterial pCO_2 from 25 to 60 mm Hg did not affect peripheral flow and resistance during perfusion

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STUDIES IN THE PHYSIOLOGY OF OXYGEN CONSUMPTION DURING CARDIOPULMONARY BYPASS*

ROY R. VETTO, LOREN C. WINTERSCHEID AND K. ALVIN MERENDINO

During clinical and experimental use of a heart lung apparatus, we have been interested in the basic physiology of perfusion, particularly with regard to oxygen consumption. During a clinically adequate perfusion, the oxygen saturation of the blood returning to the apparatus has been unduly high. This has been noted by others[1] and suggests that oxygen extraction by the perfused animals and patients has been of relatively low degree. Studies of oxygen consumption during extracorporeal circulation have been published previously.[2,3,4] The findings reported herein differ to a degree from those previously described.

METHOD

Serial oxygen uptake determinations were done in 26 animals and 13 patients during a period of cardiopulmonary bypass. In the majority of instances three determinations were performed. In animals the bypass time was standardized at 30 minutes. In patients the time of the bypass ranged from 12 to 11 minutes. Gas analyses were measured with a Van Slyke apparatus by the same technician and the quantity of perfusion was measured directly immediately following bypass. Oxygen consumption of the anesthetized dog was measured by using a fixed volume mechanical respirator and noting the difference in the partial pressures of oxygen of inspired and expired air. Room air was used for ventilation and partial pressures of oxygen were measured with a Beckman meter. Oxygen consumption was calculated assuming a respiratory quotient of one; variance with this respiratory quotient causes a negligible error in this calculation. Nine animals were used in this study. Oxygen consumption in the immediate postperfusion period was measured in 8 animals in the same manner, readings in the immediate postperfusion period being made every 30 seconds for the first 8 or 10 minutes and less frequently thereafter. A bubble oxygenator developed at the University of Washington[5,6] was used for all perfusions and all were carried out at normothermic temperatures and constant flows. In animals flow rates varied from 0.2 to 3.5 L/M²/min and in patients varied from 1.3 to 3.1 L/M²/min.

RESULTS

Oxygen consumption rates generally rose during the period of bypass but varied substantially from time to time. This is illustrated in Figure 1.

Figure 2 is a graph relating oxygen consumption to flow rate during bypass in animals. Each point represents the mean oxygen consumption for a single bypass determined from serial measurements during the course of the bypass. The mean and range of oxygen consumption of the anesthetized dog is also illustrated on the graph. Line A is a curve best representing the multiple points for mean oxygen consumption, and Line B represents values exactly 20 cc greater than those represented in Line A. During cardiopulmonary bypass

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* From the Department of Surgery, University of Washington School of Medicine, Seattle, Washington. Supported in part by the Washington State Heart Association Funds Grant #5859 9

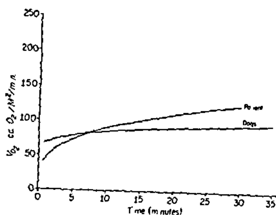


Fig 1 Change in rate of Vo_2 with time during cardiopulmonary bypass in patients and dogs perfused at a constant flow

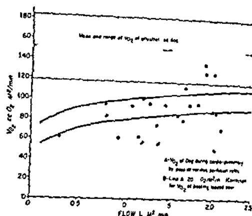


Fig 2 Vo_2 in dogs during cardiopulmo nary bypass at various perfusion rates

the oxygen consumption of the heart is not measured and since the anesthe tized dog s heart is beating and is loaded an added increment of oxygen consumption would be measured in the anesthetized dog that is not repre *sented* by Line A on the graph Oxygen consumption of the beating and loaded heart has been measured [8] and has been shown to be approximately 20 cc /M^2/min

Figure 3 is a similar graph representing data obtained in 13 patients dur ing cardiopulmonary bypass Again the lower points represent mean oxygen consumptions as measured serially during bypass We have not made direct measurements of oxygen consumption during the anesthetized state in hu man patients and have not been able to find adequate data pertaining to this subject An oxygen uptake of 150 cc /M^2/min during the basal state is considered to be normal On this graph the actual oxygen uptakes meas ured during cardiac catheterization are plotted immediately above the corresponding points of oxygen consumption during bypass Again a correc tion factor of 20 cc /M^2/min has been added to the bypass oxygen consump- tion value

One will note that there is some relationship between flow and oxygen consumption but this is certainly not a linear relationship Relatively large increases in flow produce relatively small increases in oxygen consumption

Figure 4 illustrates oxygen consumption in 8 animals in the period imme diately following bypass The mean and range of oxygen consumption are plotted against time postbypass Line A is the value for oxygen consump tion in anesthetized dogs and Line B is the value for oxygen consumption during cardiopulmonary bypass to which the 20 cc /M^2/min correction factor has been added Oxygen consumption in the first 9 minutes following the bypass is greater than normal indicating that an oxygen debt has occurred This measured oxygen debt has a mean of 237 cc with a range of 92 to 627 cc The anticipated oxygen debt based on the difference between the oxygen consumption in the anesthetized animals and the corrected oxygen con sumption during the bypass period, is 1230 cc for the 30 minute period It should be pointed out that the measurement of oxygen debt in the immediate postperfusion period is subject to error The last part of the curve is extremely shallow and very minor differences in this portion of the slope will vary the

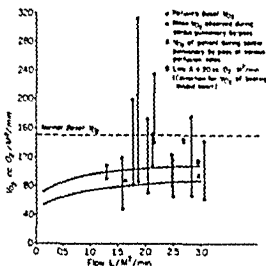


Fig 3 $\dot{V}O_2$ in patients during cardiopulmonary bypass at various perfusion rates

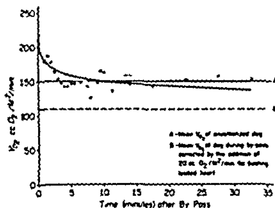


Fig 4 $\dot{V}O_2$ following cardiacpulmonary bypass in dogs

oxygen debt figures considerably Furthermore it is difficult to determine the normal oxygen consumption for the period immediately following the bypass so that one would have difficulty deciding on the actual base line of oxygen consumption for this period of time

DISCUSSION

Oxygen consumption rates in general increase during the course of a bypass and vary substantially from time to time If oxygen consumption for the whole period of bypass is calculated on the basis of a single arteriovenous difference determined just prior to the cessation of the bypass, an abnormally high result would be obtained Corrected oxygen consumptions during cardiac bypass are significantly lower than oxygen consumptions in anesthetized animals and in patients during the basal state This occurs despite an adequate source of oxygen in the perfusing blood as indicated by relatively high venous oxygen saturations Most pump oxygenator systems today produce a perfusion which is relatively pulseless In contrast, the body is accustomed to a perfusion with a systolic pressure It is conceivable that large capillary beds in the body which are relatively nonvital are simply not perfused their perfusion demanding the high systolic pressure to assure patency of the capillaries

There is some indication that a minimal oxygen uptake is required Two animals were perfused at azygos flow rates namely 0 2 and 0 3 L/M²/min and oxygen extraction by these two animals was virtually 100% Both animals survived Oxygen consumption in these 2 animals did not differ from the consumptions noted in some animals perfused at 5 times this low flow rate

Inasmuch as oxygen consumption during bypass is relatively low, one might expect that a significant oxygen debt should occur during cardiac bypass This was not substantiated by our data The mean oxygen debt was one sixth the anticipated oxygen debt and the maximal debt was one half the anticipated debt In the majority of instances the oxygen consumption rate reached pre bypass levels in the period of 10 minutes The reason for this was unknown

CONCLUSIONS

1 Oxygen consumption rates during cardiopulmonary bypass are not constant and in general tend to increase with time A single arteriovenous difference is not a reliable basis for the determination of oxygen uptake for the entire perfusion period

2 Oxygen consumption during bypass is considerably less than so called normal oxygen consumption The reason for this is unknown

3 Oxygen debts following perfusions do not appear to be equal to the anticipated oxygen debt for the perfusion period

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THE EFFECTS OF INTRAARTERIAL AIR INJECTION ON THE ELECTROENCEPHALOGRAM ELECTROCARDIOGRAM ARTERIAL AND VENOUS BLOOD PRESSURE *

S FRANK REDO

The widespread use of extracorporeal circulation techniques to bypass the heart for open cardiac surgery has increased the importance of recognizing the effects of gas emboli Microbubbles are more apt to form with certain types of oxygenators than with others [1] No matter which oxygenator is used however there is always the possibility that air will be introduced into the system through poor connections small leaks in the tubing or faulty placement of catheters In this last event the gas bubbles are likely to be fairly large and a massive amount of air may be introduced rapidly

Fries *et al* [2] injected air or oxygen into the carotid artery in dogs and found that rapid injection of 1 5 to 3 cc of gas/kg caused a marked and immediate rise in arterial blood pressure (femoral artery measurement) and pulse rate and a decrease in respiratory rate However although gradual introduction [illegible] a longer period [illegible] it was accompanied by only slight changes in vital signs

Benjamin *et al* [3] found that injection of 4 cc of air/kg into the descending aorta of dogs also produced an immediate blood pressure elevation which persisted for about 5 minutes

* From the Cornell University Medical College New York City Supported in part by the *John Polachek Foundation*

The visualization of air emboli in the retinal vessels was described by Wever[4] in 1911, but recent workers have not discussed the appearance of the eyegrounds during cerebral gas embolism studies.

Alterations of the RST segment in the electrocardiogram as a result of arterial air embolism was reported by Durant *et al*[5]

Karlson[6] indicated that after the slow injection of a total of 15 cc. of air into the internal carotid artery in dogs, the electroencephalogram was unchanged and was probably not an accurate indicator of cerebral gas embolism

The experiments here described were undertaken to study the effects of the rapid injection of air on the arterial and venous blood pressure, electroencephalogram, electrocardiogram, and cerebral and retinal vessels in the hope that a means of monitoring gas emboli in patients undergoing cardiac bypass might be found.

METHOD

Two groups of dogs were prepared In Group 1 (10 animals) the common carotid artery was exposed on the right side and ligated proximally. A polyethylene catheter on a 15 gauge needle was inserted distally. Air was rapidly injected through this in 1 to 5 cc increments

In Group 2 (15 animals) the right femoral artery was exposed and ligated distally. Through a polyethylene tube inserted proximally, air was injected rapidly into the aorta, in retrograde fashion, in 10 to 50 cc. increments. The following procedures were carried out in both groups.

A right temporoparietal craniotomy was done, allowing direct visualization of the cerebral blood vessels in the portion of brain exposed The left femoral artery and vein were cannulated to record blood pressure. Two drops each of neosynephrine (10% ophthalmic solution) and cyclogel (1% ophthalmic solution) were applied to the eyes of all animals one half hour before attempting visualization of the eyegrounds Suitable electrodes were placed for the recording of Lead II electrocardiograms and of electroencephalograms with the Grass Electroencephalograph Model III D

In all cases air injections were continued until the animal died

RESULTS

Cerebral and Retinal Vessels. In Group 1, where air was injected directly into the carotid artery, it was immediately visualized in both the cerebral and the retinal arterioles after the introduction of 0 5 to 1 0 cc. In Group 2, however, where air was injected into the femoral artery, it became evident in the cerebral and retinal vessels only after the introduction of 100 to 150 cc Air in the retinal vessels is easily seen and presents itself in the form of short columns, bubbles, or dark gaps along the red lines of blood. It moves rapidly, and what was previously a spreading red network becomes transformed into a pattern of silver The characteristic initial appearance is a gradual silvering of the blood vessels which then resemble tree branches with a thin coating of ice Later, as air almost completely replaces blood in the vessels, they become dark The disc gradually becomes blurred and changes in color from white to gray to lavender Following the injection of a small amount of air, it can be seen moving out of the arteries, being pushed on and through into the veins by the column of blood proximal to it With larger amounts, however, the blood column is stopped for a variable period, and pulsations of the vessel

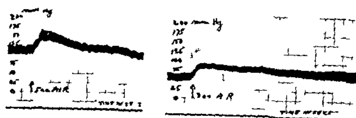


Fig 1 Arterial blood pressure tracing in Group 1 dog There is an immediate rise in pressure following the injection of 5 cc of air into the carotid artery

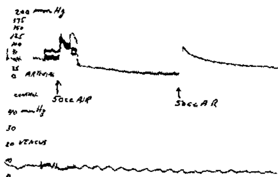


Fig 2 Arterial and venous blood pressure tracing in Group 2 dog There is an immediate rise in arterial pressure after the injection of 50 cc of air into the femoral artery Arterial tracing then resembles one of mean pressure There is no change in venous pressure

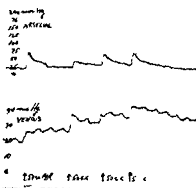


Fig 3 Arterial and venous blood pressure tracings in Group 2 dog after the injection of 200 cc of air (10 cc /kg) into femoral artery There is a simultaneous rise in both arterial and venous pressure with each additional air injection

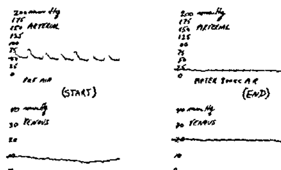


Fig 4 Arterial and venous blood pressure tracing in Group 2 dog immediately after deaths following the injection of a total of 800 cc of air Final arterial and venous pressures are the same suggesting complete arteriovenous continuity and dilatation of the capillary bed

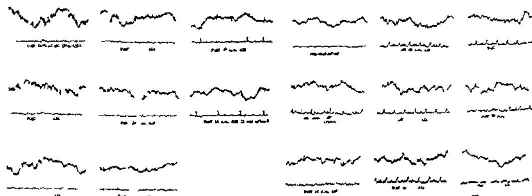


Fig 5 Electroencephalogram and electrocardiogram (Lead II) in Group 1 dog There is no apparent change in EEG In EKG P waves are low after 17 to 20 cc of air T waves are inverted after 36 cc of air have been injected into the carotid artery Bradycardia begins about 1 minute after the total of 36 cc. of air has been injected

Fig 6 Electroencephalogram and electrocardiogram (Lead II) in Group 2 dog There is no apparent change in EEG even after 170 cc of air In EKG P waves disappear after 30 cc of air has been injected and become variable appearing and disappearing as more air is injected or time passes

are evident as the blood beats against the air. Air in the cerebral vessels streams over the visible area in the arterioles and is temporarily halted as it reaches the precapillary or capillary level. However, as more air is injected or as time passes, it becomes visible in the veins, where it tends to collect in the form of large bubbles. In all instances, air was visible simultaneously in the retinal vessels and in the cerebral vessels.

Blood Pressure. *Arterial:* In Group 1 small increments of air caused an immediate rise in arterial pressure of 30 to 80 mm. Hg systolic and 20 to 50 mm. Hg diastolic. This lasted for ½ to 1 minute, following which the pressures returned to preinjection levels. After repeated injections, the increase was less marked, and the end pressure following the rise was lower than preinjection levels (Fig. 1).

The animals in Group 2 showed a similar response. However, as more air was injected, the systolic-diastolic pressure tracing was replaced by a narrow band of mean pressure, and there was a consistent drop following the peak rises (Fig. 2).

Venous: In both groups of animals there was no change in venous pressure at the outset. However, after moderately large amounts had been introduced, further injections caused simultaneous rises in both arterial and venous pressures (Fig. 3). At the time of death, venous pressure was elevated above control levels and was approximately the same as the arterial pressure (Fig. 4).

Electroencephalographic Changes. In both groups of animals changes in the EEG were not apparent until the animals were near death. In Group 1 dogs, a loss of fast activity with an increase in slow activity occurred after the injection of 40 cc. of air. The EEG showed evidence of flattening after the injection of 190 cc. of air into Group 2 animals. After these initial changes there was progressive diminution in the amplitude of the waves in both groups.

Electrocardiographic Changes. In Group 1 dogs, the earliest change noted was a lowering of the P wave after the injection of 17 to 20 cc. of air, followed by an inversion of the T wave after 30 cc., and loss of P wave with marked bradycardia following 36 cc. (Fig. 5).

Group 2 dogs revealed a loss of P wave after the injection of 30 cc. of air. Following this, the P wave became variable, appearing and disappearing as more air was injected or as time passed (Fig. 6). After 190 cc. of air, the ST segment became lower only to become markedly elevated after 230 cc.

The initial effect on rate in both groups was negligible, but with increasing amounts of air, the rate slowed markedly. In most dogs, the period of bradycardia was followed by ventricular fibrillation which continued until death.

DISCUSSION

The results indicate that the action of air is essentially the same whether it is injected into the carotid or into the femoral artery. The quantity of air necessary for visualization in the fundic vessels, however, is much greater when the femoral artery route is used. This should be expected, since air injected into this artery is distributed throughout the body and not to the vessels of the head and brain alone as is the case with intracarotid injection.

The easy recognition of air in the retinal vessels suggests that this might provide a simple way of monitoring gas emboli. However, this technique would require constant inspection of the fundi since passage through the

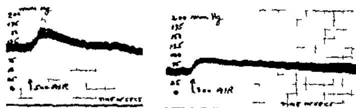
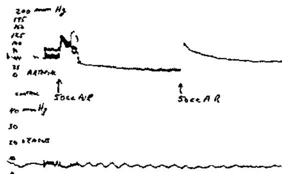


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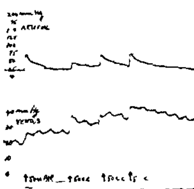


Fig 2 Arterial and venous blood pressure tracing in Group 2 dog There is an immediate rise in arterial pressure after the injection of 50 cc of air into the femoral artery Arterial tracing then resembles one of mean pressure There is no change in venous pressure

Fig 3 Arterial and venous blood pressure tracings in Group 2 dog after the injection of 200 cc of air (10 cc/kg) into femoral artery There is a simultaneous rise in both arterial and venous pressure with each additional air injection

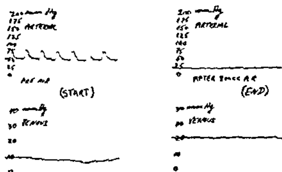
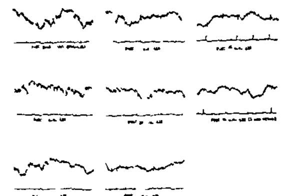


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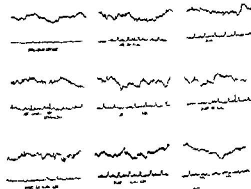


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Blood Pressure. *Arterial:* In Group 1 small increments of air caused an immediate rise in arterial pressure of 30 to 80 mm. Hg systolic and 20 to 50 mm. Hg diastolic. This lasted for ½ to 1 minute, following which the pressures returned to preinjection levels. After repeated injections, the increase was less marked, and the end pressure following the rise was lower than preinjection levels (Fig. 1).

The animals in Group 2 showed a similar response. However, as more air was injected, the systolic-diastolic pressure tracing was replaced by a narrow band of mean pressure, and there was a consistent drop following the peak rises (Fig. 2).

Venous: In both groups of animals there was no change in venous pressure at the outset. However, after moderately large amounts had been introduced, further injections caused simultaneous rises in both arterial and venous pressures (Fig. 3). At the time of death, venous pressure was elevated above control levels and was approximately the same as the arterial pressure (Fig. 4).

Electroencephalographic Changes. In both groups of animals changes in the EEG were not apparent until the animals were near death. In Group 1 dogs, a loss of fast activity with an increase in slow activity occurred after the injection of 40 cc. of air. The EEG showed evidence of flattening after the injection of 190 cc. of air into Group 2 animals. After these initial changes there was progressive diminution in the amplitude of the waves in both groups.

Electrocardiographic Changes. In Group 1 dogs, the earliest change noted was a lowering of the P wave after the injection of 17 to 20 cc. of air, followed by an inversion of the T wave after 30 cc., and loss of P wave with marked bradycardia following 36 cc. (Fig. 5).

Group 2 dogs revealed a loss of P wave after the injection of 30 cc. of air. Following this, the P wave became variable, appearing and disappearing as more air was injected or as time passed (Fig. 6). After 190 cc. of air, the ST segment became lower only to become markedly elevated after 230 cc.

The initial effect on rate in both groups was negligible, but with increasing amounts of air, the rate slowed markedly. In most dogs, the period of bradycardia was followed by ventricular fibrillation which continued until death.

DISCUSSION

The results indicate that the action of air is essentially the same whether it is injected into the carotid or into the femoral artery. The quantity of air necessary for visualization in the fundic vessels, however, is much greater when the femoral artery route is used. This should be expected, since air injected into this artery is distributed throughout the body and not to the vessels of the head and brain alone as is the case with intracarotid injection.

The easy recognition of air in the retinal vessels suggests that this might provide a simple way of monitoring gas emboli. However, this technique would require constant inspection of the fundi since passage through the

retinal vessels is rapid In addition since most present bypass methods utilize the femoral artery for perfusion inflow, moderately large amounts of air might be required before it could be visualized The fundic vessels, however, mirror the cerebral vessels, and thus retinoscopy offers a means of checking for air in the cerebral vessels

The rise that occurs with both intracarotid and intrafemoral artery injection suggests two possible mechanisms to explain the effects of air on the arterial blood pressure First, a purely central one dependent upon the effects of air on the circulatory center (perhaps secondary to local anoxia or hypoxia due to temporary cessation of the blood supply as a result of the air column) Second, a peripheral effect because of vasoconstriction at the precapillary level

Venous blood pressure is unchanged until moderate to large amounts of air have been injected After that, air introduced into the arterial side causes an immediate rise in arterial and venous pressure This suggests a complete collapse and vasodilatation of the capillary network with the establishment of a single, wide open circulation with free communication between arteries and veins This appears to occur shortly before the death of the animal and can still be demonstrated for a short time after death The changes in the electroencephalogram are not marked, even when air is injected directly into the carotid artery and can be seen in the vessels of the brain Only after the injection of fairly large amounts of air, do alterations in the EEG pattern become significant The electroencephalogram is, therefore not a sensitive monitor of cerebral gas emboli

Air injected into the carotid artery caused relatively early changes in the T and P waves of the electrocardiogram These changes may be due to a direct central effect, since the amount of air injected was not sufficient to have passed into the cerebral veins back into the right side of the heart out the pulmonary artery, back through the pulmonary veins and then into the coronary arteries as it passed out the aorta Changes in rhythm occurred in some dogs after as little as 10 cc of air had been introduced Further investigation continues on the effect on the heart of air injected into the carotid artery

When air was injected into the femoral artery, a consistent early finding was the variability of the P wave This would disappear, reappear and disappear again Changes in ST segments usually occurred later than those in the P wave These alterations are probably related to coronary artery air emboli and suggest that the sinoauricular node is markedly sensitive to hypoxia The return of the P wave is undoubtedly associated with the passage of the air column along the coronary vessels and return of circulation to the area of the S A node Disappearance recurs as air refills the coronary artery branches supplying this area

Loss of the P wave in the electrocardiogram occurs after the injection of as little as 30 cc of air into the femoral artery and may be a useful method of monitoring air embolism

SUMMARY

1 The effect of injecting air rapidly into the carotid artery in 10 dogs and into the femoral artery in 15 dogs is reported

2 Air could be seen in retinal vessels by retinoscopy in both groups but when injection was via the femoral artery moderately large amounts were necessary before visualization was possible

3 Arterial blood pressure rose immediately in both groups but fell below control levels after each rise after moderate amounts of air had been injected

4 Venous blood pressure did not change until capillary collapse occurred, after which it rose simultaneously with the arterial blood pressure with each injection When this stage was reached arterial and venous pressures were the same

5 Electroencephalographic changes did not occur until large amounts of air had been injected The EEG does not appear to be of value as a monitoring device

6 Electrocardiographic changes consisted of T and P wave alterations in both groups but were more striking in the group which was injected through the femoral artery Here loss of P wave occurred after 30 cc of air had been injected

7 P wave changes in the EKG may be a useful method of monitoring gas emboli

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A METHOD FOR THE DETECTION OF MICROBUBBLES RESULTING FROM THE PASSAGE OF BLOOD THROUGH HEART LUNG MACHINES*

RONALD TEPLER SOL GELMAN ALBERT B LOWENFELS AND JERE W LORD JR

The increasing use of extracorporeal circulation in conjunction with cardiovascular surgery has been a major advance of modern surgery The widespread use of the bubble type oxygenator by DeWall, Lillehei and their coworkers has been responsible for significant advances in open heart surgery[1] Modifications of this apparatus employing the bubble principle have been used both experimentally and clinically[2 3] Other oxygenators not employing the bubble principle have also attained wide acceptance

One of the major criteria for an efficient safe oxygenator is the absence of air emboli in the arterial perfusate[4] Evidence has been presented attributing mortality and morbidity following open heart procedures to air emboli

* From the Department of Surgery New York University Post Graduate Medical School New York City Supported in part by grants from the Cesare Barbieri Endowment and the New York Heart Association #2 66-116

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RONALD TEPPER, SOL GELMAN, ALBERT B. LOWENFELS, AND JERE W. LORD, JR.

The increasing use of extracorporeal circulation in conjunction with cardiovascular surgery has been a major advance of modern surgery. The widespread use of the bubble type oxygenator by DeWall, Lillehei and their coworkers has been responsible for significant advances in open heart surgery.[1] Modifications of this apparatus employing the bubble principle have been used both experimentally and clinically.[2, 3] Other oxygenators not employing the bubble principle have also attained wide acceptance.

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originating in the oxygenator[5, 6] This evidence has been of an indirect nature leaving open the question as to whether actual inclusions of gas were being perfused with the arterialized blood

Our group has developed a bubble monitor which allows for the direct observation of microbubbles in the arterial perfusate We have used this apparatus to evaluate the efficiency of various bubble oxygenators

METHOD

The bubble monitor is constructed of two lucite plates separated from each other by a rubber gasket 0 65 mm thick (Fig 1) The plates are held together by metal bolts and nuts Thus a thin transparent chamber is formed which allows microscopic examination of this film for foreign matter and bubbles

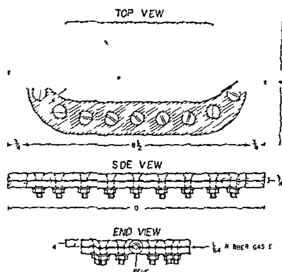


Fig 1 Diagram of the lucite bubble monitor

The oxygenators are arranged in the standard manner A blood reservoir is utilized in place of the subject undergoing perfusion Consequently fully oxygenated blood is recirculated and exposed to frothing in the oxygenation chamber The bubble monitor is connected to the arterial line by a shunt Intermittent blood samples are shunted through the monitor and then observed using a forty power wide field microscope

The following oxygenators were tested DeWall Lillehei Oxygenator, Cooley modification of DeWall Lillehei Plastic Bag Disposable Oxygenator and the Vertical Nonhelical Oxygenator The last named oxgenator consists of a bubbling chamber within a vertical arterial reservoir It is essentially a Cooley oxgenator without a helix

Human blood and fresh canine blood were circulated Bubble counts were obtained periodically during perfusion at constant O_2 and blood flow rates in order to plot the number of bubbles present against time of perfusion In another series of runs the O_2 flow was varied while the blood flow remained constant A sigma pump was used throughout

RESULTS

When bubbles were present they were easily observed They ranged in size from 20 to 125 microns

The plastic bag disposable oxygenator was evaluated utilizing a blood flow rate of 1 000 cc /min and an oxygen flow of 3 L/min Recirculation of the

fully oxygenated blood for a period of 2 hours through the oxygenators under these conditions failed to produce detectable bubbles

Figure 2 illustrates the number of bubbles escaping from each of the other oxygenators studied The blood flow rate was constant at 1,000 cc/min The major variable was found to be the oxygen flow rate Increasing the oxygen flow rate resulted in an increased number of bubbles passing through the arterial line The vertical nonhelical oxygenator produced approximately 150 bubbles per microscopic field at 3 liters of oxygen flow per minute The DeWall Lillehei oxygenator produced an occasional bubble at 3 liters of oxygen per minute Increasing the oxygen flow resulted in an increase in the number of bubbles observed The Cooley oxygenator for any given oxygen flow rate allowed 2 to 4 times the number of bubbles to pass through as did the DeWall Lillehei apparatus Lowering the level in the arterial reservoir of the Cooley oxygenator increased the number of bubbles present As long as the arterial and O_2 flow rates were constant the number of bubbles did not vary significantly with the time in any of the oxygenators tested

Preliminary studies of actual perfusions, in which desaturated venous blood is oxygenated, revealed no microbubbles passing through the DeWall Lillehei or the Cooley oxygenators The vertical nonhelical oxygenator permits many bubbles to reach the animal

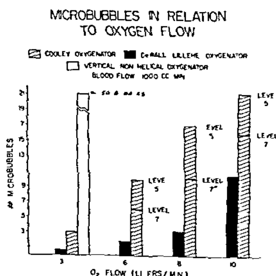


Fig 2 The number of microbubbles increases with the increase in the O_2 flow Lowering the level of the arterial reservoir in the Cooley oxygenator increases the number of bubbles noted The nonhelical oxygenator was discarded after the initial testing

DISCUSSION

The morbid effects of bubble formation have frequently been cited as limiting the application of various oxygenators We have demonstrated that under certain conditions microbubbles do form within the blood passing through these machines

We have eliminated the possibility of the monitor forming these bubbles The bubble monitor will withstand air pressure of 15 pounds per square inch without evidence of leaks The fluid flow through the monitor is nonturbulant thus cavitation does not occur[7] Saturated blood has been recirculated for several hours through the monitor without bubble formation

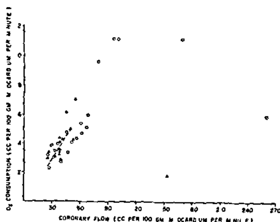


Fig 2 Coronary venous flow *versus* ventricular oxygen consumption

sinus drainage was collected in a second calibrated reservoir Blood from both coronary venous reservoirs was returned to the pulmonary circulation by an accessory pump Continuous recording of central aortic pressure, left and right ventricular pressures and cardiac rate was obtained

As soon as stability of the preparation was evident multiple simultaneous determinations were obtained for coronary blood flow, right and left ventricular and aortic pressures, cardiac rate and coronary A V oxygen difference Blood gas analyses were performed by standard techniques on a Van Slyke manometric apparatus

At the conclusion of the study the animal was sacrificed the heart opened *in situ* and the position of the coronary sinus catheter and suture ligature checked The ventricles were excised and weighed according to the method of Herrmann [4]

Calculations were made of ventricular work, efficiency, myocardial oxygen consumption and coronary vascular resistance

OBSERVATIONS

The coronary blood flows per minute through the right and left sides of the heart differed in all experiments They varied from 23 to 154 ml /100 gm of myocardium for the left ventricle (mean 48 ml) and from 27 to 260 ml /100 gm of myocardium for the right side of the heart (mean 67 ml) The volume of coronary flow (CF) showed a good correlation with oxygen consumption in both ventricles (Fig 2) Right and left ventricular coronary flows were found to be related (Fig 3) No relationship was found between CF and efficiency, heart rate or mean systolic ventricular pressure Coronary flow per ventricle showed a fair correlation with mean aortic pressure (Fig 4) However, when CF was calculated in terms of ml /100 gm of myocardium per minute, there was no correlation of CF with mean aortic pressure Right ventricular CF/100 gm of myocardium showed a good correlation with work per minute (Fig 5) This could not be demonstrated for the left ventricle, although there was a fair correlation between work per minute of the left ventricle and CF for the total left ventricle (Fig 6)

Coronary vascular resistances (CVR) were calculated separately for the two ventricles The right ventricular CVR varied from 0 18 to 2 30 mm Hg/ml / 100 gm of myocardium (mean 1 52) The left ventricular CVR varied from 0 28 to 2 70 mm Hg/ml /100 gm of myocardium (mean 1 83) Left ventricular CVR showed a fair correlation with work per minute (Fig 7) and with ventricular efficiency (Fig 8) No such relation could be demonstrated in the

Fig 3 Coronary venous flow, right ventricle *versus* left ventricle

Fig 4 Mean aortic pressure *versus* coronary venous flow per ventricle

Fig 5 Right ventricular coronary venous flow (ml /*100 gm of myocardium*/min) *versus* right ventricular work

Fig 6 Left ventricular coronary venous flow (ml /*ventricle*/min) *versus* left ventricular work

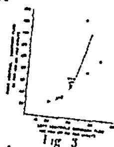


Fig 3

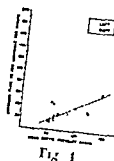


Fig 4



Fig 5



Fig 6

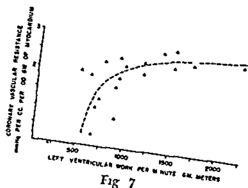


Fig 7

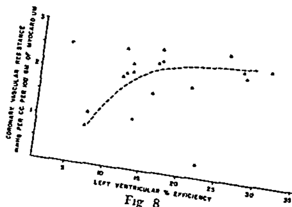


Fig 8

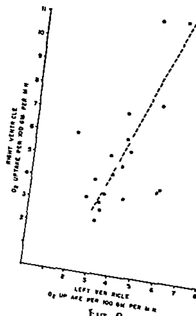


Fig 9

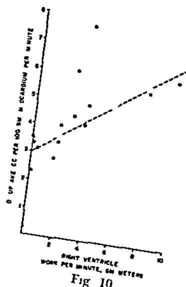


Fig 10

Fig 7 Left ventricular work *versus* left ventricular coronary vascular resistance

Fig 8 Left ventricular efficiency *versus* left ventricular coronary vascular resistance

Fig 9 Oxygen consumption right ventricle *versus* left ventricle

Fig 10 Right ventricular oxygen consumption *versus* right ventricular work

right ventricle. Coronary vascular resistance also could not be shown to correlate with oxygen consumption, heart rate, nor mean ventricular systolic pressure.

The oxygen consumption (Vo_2) per minute for the right and left sides also differed. The left ventricular Vo_2 ranged from 1.9 to 8.3 ml./100 gm. of myocardium/min. (mean 4.6 ml.). The Vo_2 of the right ventricle ranged from 2.3 to 11.2 ml./100 gm/min. (mean 5.7 ml.). Oxygen consumption, as noted above, showed a good correlation with coronary flow (Fig. 2). Right and left ventricular oxygen consumptions showed a fair correlation with each other (Fig. 9). *In the right ventricle there was a correlation between Vo_2 and both work per minute and stroke volume (Fig. 10, 11).* No such correlation could be demonstrated for the left ventricle. Only a rough relationship was found between Vo_2 and heart rate. With heart rates over 100 per minute most of the Vo_2 values were above 4 ml./100 gm. of myocardium/min. while with heart rates under 100/min. most of the Vo_2 values were less than 4 ml./100 gm. of myocardium/min. No correlation was found between Vo_2 and CVR, mean systolic ventricular pressure, mean aortic pressure, nor efficiency.

Intraventricular pressures and work. The mean right ventricular systolic pressure varied from zero to 21 mm. Hg (mean 6.2 mm. Hg). The mean systolic left ventricular pressure varied from 41 to 149 mm. Hg (mean 81 mm. Hg). The calculated work per minute of the right ventricle ranged from zero

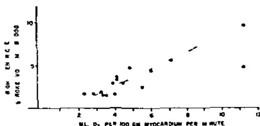


Fig. 11

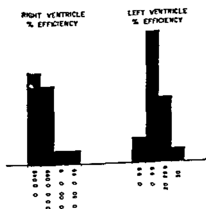


Fig. 12

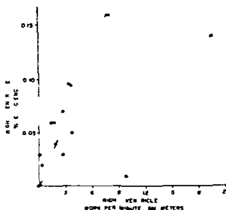


Fig. 13

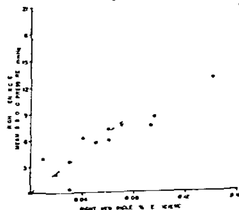


Fig. 14

Fig. 11. Right ventricular oxygen consumption *versus* right ventricular stroke volume.
Fig. 12. Distributions of right and left ventricular efficiencies.
Fig. 13. Right ventricular work *versus* right ventricular efficiency.
Fig. 14. Right ventricular efficiency *versus* right ventricular mean systolic pressure.

to 19.3 gram-meters per minute (mean 3.8 gram-meters). The calculated work of the left ventricle ranged from 522 to 2230 gram-meters per minute (mean 1052 gram meters).

The *efficiency* of the right ventricle varied from zero to 0.16% (mean 0.06%). The left ventricular efficiency varied from 7.0 to 31.5% (mean 17.3%). The distribution of ventricular efficiency is shown in Fig. 12. The right ventricular efficiency correlated well with both work per minute and mean ventricular systolic pressure (Fig. 13, 14), although these relations could not be demonstrated for the left ventricle.

DISCUSSION

The volume of the coronary venous return of the right heart is very similar to that of the left heart in most cases. This might be due to equilibration of coronary flows by shunting of a portion of the left ventricular coronary venous return away from the coronary sinus through extensive communications between the left ventricular vascular bed and the anterior cardiac veins and the minute myocardial vessels. It is clear that a means must be found to quantitate the degree of mixing under varying physiologic conditions; the mixing is probably small however, since there is a significant difference in virtually every case between the right and left ventricular values for arteriovenous oxygen difference and coronary vascular resistance.

A strong correlation of coronary flow with myocardial oxygen consumption has been reported previously,[5, 6] as has the lack of correlation of mean aortic flow with mean aortic pressure *in vivo*.[6] The fact that coronary flow per 100 grams of myocardium is greater through the bypassed right ventricle than through the working left ventricle might suggest a causal relationship with decreased intraventricular systolic pressure. However, the data indicate that mean intraventricular systolic pressure has no demonstrable relationship to coronary flow or to coronary vascular resistance in either ventricle. This is contrary to classical opinion as is the lack of correlation between coronary flow and heart rate.

Recognizing the limitations of the method, by far the most interesting finding in this study is that the right ventricle, doing essentially no external work, uses approximately as much oxygen as the normally working left ventricle. Thus the energy expenditure of the two ventricles is of the same magnitude. This might be explained by the following concept: a ventricle must use a certain minimum amount of oxygen for muscular contraction *per se*, regardless of work, at least this is so in the range of work performed by the heart in these experiments. That the external work is largely dependent upon the diastolic volume is suggested by the fact that with proportionate increases in right ventricular diastolic volumes the external work and efficiency increase *pari passu*. The data do not afford any indication of what factors establish this minimum level of energy expenditure in the contracting bypassed ventricle.

SUMMARY

1. A method has been described whereby ventricular dynamics and coronary blood flows for the right and left ventricles can be studied separately and simultaneously. This method was applied to a preparation in which only one ventricle had a significant work load.

right ventricle Coronary vascular resistance also could not be shown to correlate with oxygen consumption heart rate nor mean ventricular systolic pressure

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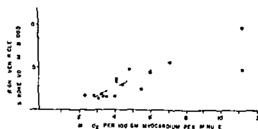


Fig 11

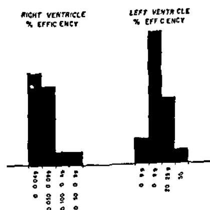


Fig 12

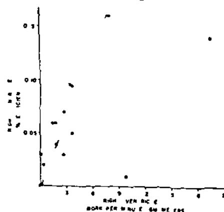


Fig 13

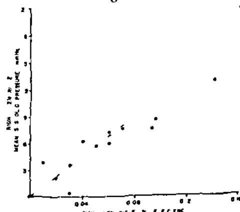


Fig 14

Fig 11 Right ventricular oxygen consumption *versus* right ventricular stroke volume
Fig 12 Distributions of right and left ventricular efficiencies
Fig 13 Right ventricular work *versus* right ventricular efficiency
Fig 14 Right ventricular efficiency *versus* right ventricular mean systolic pressure

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ing in this study is that the right
uses approximately as much oxygen as the normally working left ventricle Thus the energy expenditure of the two ventricles is of the same magnitude This might be explained by the following concept a ventricle must use a certain minimum amount of oxygen for muscular contraction *per se*, regardless of work at least this is so in the range of work performed by the heart in these experiments That the external work is largely dependent upon the diastolic volume is suggested by the fact that with proportionate increases in right ventricular diastolic volumes the external work and efficiency increase *pari passu* The data do not afford any indication of what factors establish this minimum level of energy expenditure in the contracting bypassed ventricle

SUMMARY

1 A method has been described whereby ventricular dynamics and coronary blood flows for the right and left ventricles can be studied separately and simultaneously This method was applied to a preparation in which only one ventricle had a significant work load

right ventricle Coronary vascular resistance also could not be shown to correlate with oxygen consumption heart rate, nor mean ventricular systolic pressure

The oxygen consumption (Vo_2) per minute for the right and left sides also differed The left ventricular Vo_2 ranged from 1 9 to 8 3 ml /100 gm of myocardium/min (mean 4 6 ml) The Vo_2 of the right ventricle ranged from 2 3 to 11 2 ml /100 gm/min (mean 5 7 ml) Oxygen consumption as noted above showed a good correlation with coronary flow (Fig 2) Right and left ventricular oxygen consumptions showed a fair correlation with each other (Fig 9) In the right ventricle there was a correlation between Vo_2 and both work per minute and stroke volume (Fig 10 11) No such correlation could be demonstrated for the left ventricle Only a rough relationship was found between Vo_2 and heart rate With heart rates over 100 per minute most of the Vo_2 values were above 1 ml /100 gm of myocardium/min while with heart rates under 100/min most of the Vo_2 values were less than 4 ml /100 gm of myocardium/min No correlation was found between Vo_2 and CVR mean systolic ventricular pressure mean aortic pressure, nor efficiency

Intraventricular pressures and work The mean right ventricular systolic pressure varied from zero to 21 mm Hg (mean 6 2 mm Hg) The mean systolic left ventricular pressure varied from 41 to 119 mm Hg (mean 81 mm Hg) The calculated work per minute of the right ventricle ranged from zero

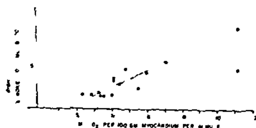


Fig 11

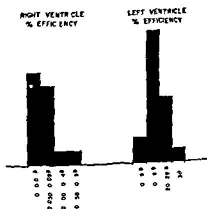


Fig 12

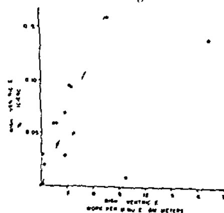


Fig 13

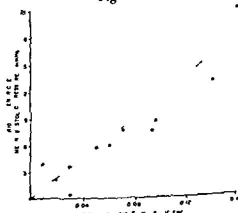


Fig 14

Fig 11 Right ventricular oxygen consumption *versus* right ventricular stroke volume
Fig 12 Distributions of right and left ventricular efficiencies
Fig 13 Right ventricular work *versus* right ventricular efficiency
Fig 14 Right ventricular efficiency *versus* right ventricular mean systolic pressure

to 19 3 gram meters per minute (mean 3.8 gram meters). The calculated work of the left ventricle ranged from 522 to 2230 gram meters per minute (mean 1052 gram meters).

The *efficiency* of the right ventricle varied from zero to 0.16% (mean 0.06%). The left ventricular efficiency varied from 7.0 to 31.5% (mean 17.3%). The distribution of ventricular efficiency is shown in Fig. 12. The right ventricular efficiency correlated well with both work per minute and mean ventricular systolic pressure (Fig. 13, 14), although these relations could not be demonstrated for the left ventricle.

DISCUSSION

The volume of the coronary venous return of the right heart is very similar to that of the left heart in most cases. This might be due to equilibration of coronary flows by shunting of a portion of the left ventricular coronary venous return away from the coronary sinus through extensive communications between the left ventricular vascular bed and the anterior cardiac veins and the minute myocardial vessels. It is clear that a means must be found to quantitate the degree of mixing under varying physiologic conditions; the mixing is probably small however, since there is a significant difference in virtually every case between the right and left ventricular values for arteriovenous oxygen difference and coronary vascular resistance.

A strong correlation of coronary flow with myocardial oxygen consumption has been reported previously,[5, 6] as has the lack of correlation of mean aortic flow with mean aortic pressure *in vivo*.[6] The fact that coronary flow per 100 grams of myocardium is greater through the bypassed right ventricle than through the working left ventricle might suggest a causal relationship with decreased intraventricular systolic pressure. However, the data indicate that mean intraventricular systolic pressure has no demonstrable relationship to coronary flow or to coronary vascular resistance in either ventricle. This is contrary to classical opinion as is the lack of correlation between coronary flow and heart rate.

Recognizing the limitations of the method, by far the most interesting finding in this study is that the right ventricle, doing essentially no external work, uses approximately as much oxygen as the normally working left ventricle. Thus the energy expenditure of the two ventricles is of the same magnitude. This might be explained by the following concept: a ventricle must use a certain minimum amount of oxygen for muscular contraction *per se*, regardless of work, at least this is so in the range of work performed by the heart in these experiments. That the external work is largely dependent upon the diastolic volume is suggested by the fact that with proportionate increases in right ventricular diastolic volumes the external work and efficiency increase *pari passu*. The data do not afford any indication of what factors establish this minimum level of energy expenditure in the contracting bypassed ventricle.

SUMMARY

1. A method has been described whereby ventricular dynamics and coronary blood flows for the right and left ventricles can be studied separately and simultaneously. This method was applied to a preparation in which only one ventricle had a significant work load.

2 It was demonstrated that there is a good correlation between coronary blood flow and myocardial oxygen consumption but not between coronary blood flow and mean aortic pressure

3 No relationship could be demonstrated between mean intraventricular systolic pressure and coronary flow or coronary vascular resistance

4 The bypassed right ventricle doing virtually no work consumed approximately as much oxygen as the left ventricle which was doing work within a physiologic range

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MYOCARDIAL METABOLISM DURING PROLONGED ASYSTOLE AND CARDIOPULMONARY BYPASS *

PHILLIP M IKINS DANIEL M ENERSON AND C BARBER MUELLER

Information concerning altered myocardial metabolism during elective cardiac arrest is important in defining optimum conditions for open heart surgery and the maximum safe time of arrest Such metabolic measurements must be expressed as utilization—the product of coronary flow and the arterio venous concentration difference of a given metabolite The mechanical difficulties of measuring coronary flow during cardiopulmonary bypass using the mechanical pump oxygenator are considerable and elective cardiac arrest introduces still further technical problems which make accurate measurement difficult This paper reports our studies of the utilization and production of several metabolites by the myocardium during conditions of cardiopulmonary bypass and elective cardiac arrest We are also reporting on a new method of employing the nitrous oxide technique [1, 2] to measure coronary blood flow by the introduction of nitrous oxide and oxygen directly into the pump oxygenator

METHOD

A series of 43 mongrel dogs of 17 to 30 kg was used in the initial group of experiments (Series A) A transsternal bilateral thoracotomy was performed and the pericardium was opened A De Wall bubble oxygenator was used with venous return catheters in the superior and inferior vena cavae Arterial delivery from the pump oxygenator was divided with a stainless steel Y tube *into the left subclavian artery for systemic* delivery and by a special needle or

* From the Department of Surgery State University of New York Upstate Medical Center Syracuse N Y Supported by U S Public Health Service Grant #H 3287

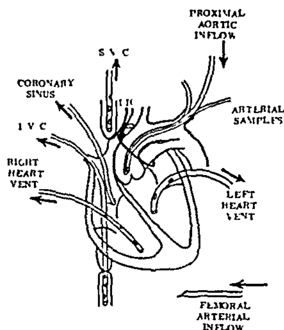


Fig 1 Method of preparation used in Series B

catheter into the proximal aorta for coronary delivery Cardiopulmonary bypass was instituted the aorta was cross clamped between the two points of arterial delivery and the nitrous oxide oxygen mixture was introduced into the oxygenator At this time simultaneous blood samples were drawn from the proximal aorta and coronary sinus The heart was arrested by the injection of potassium chloride into the proximal aorta at the same time that nitrous oxide was again introduced into the pump oxygenator and arterial and venous samples were again drawn from the coronary circulation Nitrous oxide oxygen and carbon dioxide were measured by gasometric means in the Van Slyke apparatus Measurements were also made of whole blood pH, glucose lactate pyruvate and ketones

In Series B these same metabolic measurements were made but the physiological preparation was altered in order to check the reliability of the nitrous oxide coronary flow determinations used in Series A On 8 dogs nitrous oxide coronary flow measurements were made before and after arrest while the proximal aortic delivery was maintained at a constant rate by an independent sigmamotor pump instead of being delivered from one limb of a Y connector The pericardium was closed during the metabolic studies to minimize the loss of nitrous oxide The pulmonary artery was occluded to isolate the right and left cardiac chambers which were drained by separate siphons Coronary venous samples were collected simultaneously from a coronary sinus catheter and from the right heart siphon Aortic pressures were continuously measured in both the proximal and distal aorta The pump oxygenator was started and the rate of systemic arterial perfusion was adjusted to provide a normal arterial pressure After aortic cross clamping the proximal and distal aortic pressures were equalized by changing the proximal aortic flow During perfusion normothermia was maintained by the incorporation of a heat exchanger in the pump oxygenator system

In most of the animals in each series after completion of cardiac arrest and the necessary sampling the aorta was disoccluded cardiac rhythm was restored and perfusion was slowly discontinued in order to judge the ability of the heart to maintain the circulation

2 It was demonstrated that there is a good correlation between coronary blood flow and myocardial oxygen consumption but not between coronary blood flow and mean aortic pressure

3 No relationship could be demonstrated between mean intraventricular systolic pressure and coronary flow or coronary vascular resistance

4 The bypassed right ventricle doing virtually no work consumed approximately as much oxygen as the left ventricle which was doing work within a physiologic range

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PHILLIP M IKINS DANIEL M ENERSON AND C BARBER MUELLER

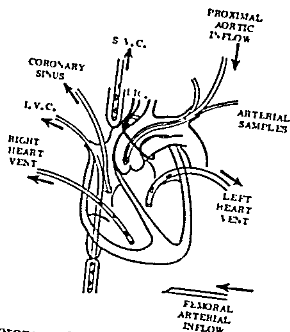
Information concerning altered myocardial metabolism during elective cardiac arrest is important in defining optimum conditions for open heart surgery and the maximum *safe time of arrest* Such metabolic measurements must be expressed as utilization—the product of coronary flow and the arteriovenous concentration difference of a given metabolite The mechanical difficulties of measuring coronary flow during cardiopulmonary bypass using the mechanical pump oxygenator are considerable, and elective cardiac arrest introduces still further technical problems which make accurate measurement difficult This paper reports our studies of the utilization and production of several metabolites by the myocardium during conditions of cardiopulmonary bypass and elective cardiac arrest We are also reporting on a new method of employing the nitrous oxide technique [1, 2] to measure coronary blood flow by the introduction of nitrous oxide and oxygen directly into the pump oxygenator

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In Series B these same metabolic measurements were made but the physiological preparation was altered in order to check the reliability of the nitrous oxide coronary flow determinations used in Series A On 8 dogs nitrous oxide coronary flow measurements were made before and after arrest while the proximal aortic delivery was maintained at a constant rate by an independent sigmamotor pump instead of being delivered from one limb of a Y-connector. The pericardium was closed during the metabolic studies to minimize the loss of nitrous oxide The pulmonary artery was occluded to isolate the right and left cardiac chambers, which were drained by separate siphons Coronary venous samples were collected simultaneously from a coronary sinus catheter and from the right heart siphon Aortic pressures were continuously measured in both the proximal and distal aorta The pump oxygenator was started and the rate of systemic arterial perfusion was adjusted to provide a normal arterial pressure After aortic cross clamping the proximal and distal aortic pressures were equalized by changing the proximal aortic flow During perfusion normothermia was maintained by the incorporation of a heat exchanger in the pump oxygenator system

In most of the animals in each series, after completion of cardiac arrest and the necessary sampling the aorta was disoccluded, cardiac rhythm was restored and perfusion was slowly discontinued in order to judge the ability of the heart to maintain the circulation.

RESULTS

In the initial group of experiments (Series A) the oxygen utilization of the myocardium in the open chest dog varied from 4 9 to 6 9 cc /100 gm left ventricle/min The mean value obtained from the 43 animals was 5 9 cc /100 gm left ventricle/min During cardiopulmonary bypass the beating heart relieved of its external work load used 2 1 cc /100 gm left ventricle/min and the heart in sustained arrest used 2 8 cc /100 gm left ventricle/min The utilization of glucose by the normal heart was 3 4 mg /100 gm ventricle/min This increased to 6 0 mg during bypass and to 13 5 mg during cardiac arrest Changes in the utilization of lactate and pyruvate (Fig 2) were somewhat less consistent than those noted with glucose Generally as the arterial concentration of lactate and pyruvate increased the myocardium utilized more and more of these compounds However during prolonged perfusion the coronary sinus concentration of lactate exceeded the arterial concentration suggesting that lactate was no longer a substrate, but had become a product of the metabolic activity At such periods as this the systemic arterial pH was noted to decrease gradually

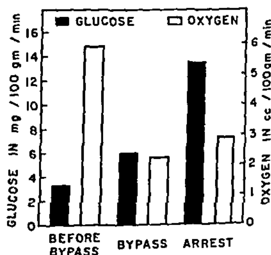


Fig 2 Oxygen and glucose utilization

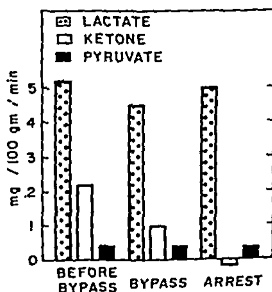


Fig 3 Lactate ketone and pyruvate utilization

Table 1. Apparent Changes in Coronary Flow During KCL Arrest Series B

Dog No	Fixed Arterial Delivery in cc/min	Pre Arrest Flow in cc/100 Gm/min N_2O Method: Coronary Sinus	Right Heart	Flow During Arrest in cc/100 Gm/min N_2O Method: Coronary Sinus	Right Heart
60	—	45.3		15.3	
62	115	62.7		41.0	
63	196	106.6		163.8	
64	185	126.8		109	
65	113	191		136	
73	144	60.2	57.8	97.6	62
74	144	85.8	48.1	97	58.6
79	144	81.8	60.7	47.4	43.1

The same general metabolic findings were obtained in Series B but the study is too small to draw statistical conclusions. There is an apparent increase in coronary flow with cardiac arrest in some animals and a decrease in others despite the experimental condition of fixed coronary delivery. Arterial shunts into the coronary sinus or reflux at the aortic or pulmonary valves may be some of the features which contribute to the artefacts in these measurements. For example, the results obtained in dog 73, Table 1, suggest that the apparent increase in coronary flow with cardiac arrest may be an artefact produced by sampling the coronary sinus.

Discussion

The marked reduction in oxygen consumption by the myocardium during cardiopulmonary bypass is a reflection of the decreased work load of the heart during bypass. The fact that there is no significant further change during induced cardiac arrest suggests that the surgical advantage of elective cardiac arrest is not one of decreased oxygen utilization. The concomitant increase in glucose utilization during bypass and during elective arrest is not easily explained at present. This change does not appear to be associated with the usual course of anaerobic glucose metabolism since an increase in the production of lactic acid by the myocardium was not detected except during perfusions of an hour or more. The metabolism of the myocardium differs from skeletal muscle in that it normally utilizes lactic acid as a substrate, however, during protracted perfusions, cardiac metabolism appears to resemble skeletal muscle more closely than usual and produces lactic acid as a metabolic end product.

The method used to measure coronary flow during bypass and arrest undoubtedly produces artefacts in the metabolic data. Gregg[3] catheterizes the left coronary artery and assumes that there is no significant contamination of the coronary sinus by right coronary blood. The method used by Bing to study the arrested heart[4, 5] assumes a quantitative delivery into the coronary arteries through a balloon catheter which occludes the aortic ring. Balloon

RESULTS

In the initial group of experiments (Series A) the oxygen utilization of the myocardium in the open chest dog varied from 4 9 to 6 9 cc /100 gm left ventricle/min The mean value obtained from the 43 animals was 5 9 cc./100 gm left ventricle/min During cardiopulmonary bypass, the beating heart relieved of its external work load, used 2 1 cc /100 gm left ventricle/min and the heart in sustained arrest used 2 8 cc /100 gm left ventricle/min The utilization of glucose by the normal heart was 3 4 mg /100 gm ventricle/min This increased to 6 0 mg during bypass and to 13 5 mg during cardiac arrest Changes in the utilization of lactate and pyruvate (Fig 2) were somewhat less consistent than those noted with glucose Generally, as the arterial concentration of lactate and pyruvate increased, the myocardium utilized more and more of these compounds However, during prolonged perfusion, the coronary sinus concentration of lactate exceeded the arterial concentration suggesting that lactate was no longer a substrate, but had become a product of the metabolic activity At such periods as this, the systemic arterial pH was noted to decrease gradually

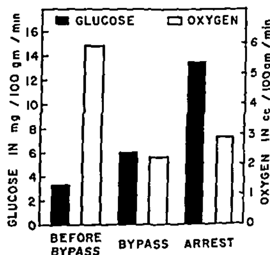


Fig 2 Oxygen and glucose utilization

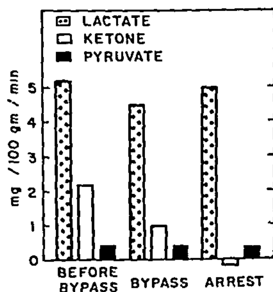


Fig 3 Lactate ketone and pyruvate utilization

Table I Apparent Changes in Coronary Flow During KCl Arrest Series B

Dog No.	Fixed arterial delivery in cc/min	Pre-arrest flow in cc/100 Gm/min N_2O method: Coronary sinus	Right heart	Flow during arrest in cc/100 Gm/min N_2O method: Coronary sinus	Right heart
60	—	[illegible]		[illegible]	
62	113	62.7		41.0	
63	196	106.6		103.8	
64	18[illegible]	120.8		109	
65	143	191		136	
73	141	60.2	7.8	97.6	62
74	111	8 8	18.1	97	38.6
79	111	81.8	60.[illegible]	47.1	43.1

The same general metabolic findings were obtained in Series B but the study is too small to draw statistical conclusions. There is an apparent increase in coronary flow with cardiac arrest in some animals and a decrease in others despite the experimental condition of fixed coronary delivery. Arterial shunts into the coronary sinus or reflux at the aortic or pulmonary valves may be some of the features which contribute to the artefacts in these measurements. For example the results obtained in dog 73 Table I suggest that the apparent increase in coronary flow with cardiac arrest may be an artefact produced by sampling the coronary sinus.

DISCUSSION

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occlusion of the aortic valve has not produced satisfactory occlusion in our hands with a normal rate of coronary perfusion Collection of venous drainage of the coronary system[6-7] is not a sufficiently exact method of coronary flow measurement The basic technique of metabolic and coronary flow measurement introducing nitrous oxide into the pump oxygenator may be a useful means of studying myocardial metabolism under a variety of physical and chemical states that may be selected by the investigator While basically simple in application it still requires further standardization with existent methods of measurement

SUMMARY

1 Oxygen utilization by the dog myocardium decreases markedly during cardiopulmonary bypass Cardiac arrest produces little further change

2 Glucose utilization by the dog myocardium increases markedly during bypass and increases still further during elective cardiac arrest

3 To obtain these coronary flow and metabolite data a method to measure coronary blood flow using the pump oxygenator is described

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PATHOLOGICAL CHANGES AFTER PARTIAL AND TOTAL CARDIOPULMONARY BYPASS IN HUMANS AND ANIMALS*

PETER K KOTTMEIER JANIS ADAMSONS JACKSON H STUCKEY
MELVIN M NEWMAN AND CLARENCE DENNIS

The use of extracorporeal units in the past few years has been accompanied by reports of a wide variety of complications Difficulties have been attributed to air fibrin and antifoam embolism as well as to profound metabolic changes Hemolysis oxygen toxicity and other factors have also caused concern It is difficult to differentiate on postmortem examination between changes resulting from the perfusion and those which were present at the

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time of the bypass In view of these considerations, it appeared to us that a review of the pathologic changes to be found in dogs at various periods after total cardiopulmonary bypass might be of value

METHOD

Mongrel dogs of approximately 20 kg body weight were placed on total bypass utilizing the machine which has been described by this laboratory.[1] In some experiments, a sigmamotor pump was used instead of our modified Dale Schuster pump In 9 animals, one hour perfusions were performed to establish that we could obtain consistent survivals In others, much longer *periods of bypass, with ventriculotomy were used in connection with studies* on the path of the conduction system; all of these were sacrificed at once. An attempt was made to maintain the body temperature between 95 and 99°F., but in the longer procedures with cardiotomy, it frequently dropped to the neighborhood of 92°F. Pure oxygen was used for the Jefferson respirator and the oxygenator in each instance In certain cases the respirator was operated throughout the period of bypass, and in others it was not. The details of the operations are presented in Tables 1, 2, and 3.

Table 1

9 DOGS ON 60 MIN TOTAL BYPASS

NO	TIME SACRIFICED POSTOP	FLOW ML./KG	M AORTIC BP	PROTAMINE TITER		ART O_2
784	postop	77	77	donor 90	End 50	89 4%
801	postop	65	80	90	80	89.5%
693	1st da	77	70	90	50	92.3%
776	7th da	63	83	90	60	85 6%
772	8th da	70	75	90	80	87 1%
749	8th da	80	95	90	60	87 6%
729	10th da	70	82	80	50	86 4%
794	180th da	100	70	85	70	
725	180th da	66	57	90	70	97 0%
Lowest		63	57	60	50	85 6%
Highest		100	95	90	80	97 0%
Average		76	75	85	63	89 4%

Flows in ml/min per kg body weight

M Aortic BP—mean aortic blood pressure

Protamine titer in μg/ml

Art O_2—arterial oxygen saturation

Dog #776 and 725 were kept on respiration during bypass

In this group cardiac fibrillation was used and the left auricle as well as the right was drained into the oxygenator Blood was returned from the pump oxygenator via the subclavian artery

Table 2

8 DOGS ON 60 TO 138 MIN. TOTAL BYPASS							
NO.	TIME SACRIFICED	FLOW ML./KG.	M. AORTIC BP	PLASMA HB	ANTI-FOAM	RESPIRATOR	BYPASS TIME MIN.
1093	postop	80	79	240	Yes	No	75
1080	postop	100	85	290	Yes	Yes	60
833	postop	80	73	208	No	No	65
983	postop	80	69	242	No	Yes	90
1062	postop	70	55		No	Yes	138
59	postop	80	52	306	Yes	Yes	60
75	postop	70	68	115	Yes	No	120
68	postop	80	88	165	Yes	No	90
Lowest		70	52	115			60
Highest		100	88	306			138
Average		80	71	233	3 without	4 without	87

The first 2 dogs had right ventriculotomy, the remaining 6 bilateral ventriculotomy. Heart was not fibrillated. High plasma hemoglobin due to use of suction blood during ventriculotomy.

Table 3

3 DOGS ON 90 MIN. TOTAL BYPASS					
NO.	TIME SACRIFICED POSTOP	FLOW ML./KG.	M. AORTIC BP	ANTIFOAM	RESPIRATOR
668	180th da.	100	70	Yes	Yes
656	180th da.	80	50	Yes	Yes
450	180th da.	80	120	Yes	Yes
Average	180th da.	86	80	Yes	Yes

Dennis oxygenator and bubble trap with sigmamotor pump. Heart fibrillated.

RESULTS

Postmortem examination showed visceral congestion in all groups to a varying degree, mainly in liver and spleen. No correlation between factors like blood flow, arterial blood pressure, protamine titers, etc., and the degree of congestion could be demonstrated. In an occasional animal, hepatic perivascular hemorrhage was seen (see Fig. 1).

The most striking finding was the presence of "pulmonary collapse"[2] and peribronchial hemorrhage in animals included in Table 1. Since the dogs showing pulmonary changes in Table 1 were not supported by artificial respiration during bypass, half of the dogs included in Table 2 was supported by a Jefferson respirator with a respiration rate from 10 to 15/min. and reduced oxygen flow and the other half was not kept on the respirator. In this

group only animals not supported by respiration showed pulmonary changes such as peribronchial hemorrhage (see Fig 2) Since the continued inflation of lungs during bypass we have not seen any clinical evidence of pulmonary collapse or hemorrhage in dogs[3]

In 2 animals perfused for 60 minutes with a sigmamotor pump and the Dennis oxygenator emboli were found at the end of the bypass with the animals still alive The emboli were found in the mesenteric artery in one and in the left anterior descending coronary artery in both The material found in the vessels is similar to that seen in parts of the extracorporeal circuit especially the oxygenator Fat stain was negative (see Fig 3)

Autopsies of the survivors listed in Table 3 which were perfused under similar circumstances as the animals with the gross emboli failed to show any evidence of infarction

To rule out the possibility that the material found in the vessels could represent antifoam 3 animals included in Table 2 were perfused without antifoam in the circuit (see Figs 4 and 5) Embolic material was found in the renal arterioles in animals of both groups



Fig 1 Dog No 983 Table 2 Hepatic perivascular hemorrhage



Fig 2 Dog No 784 Table 1 Marked peribronchial hemorrhage

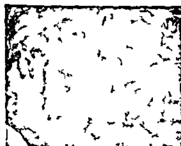


Fig 3 Material found in the oxygenator at the end of perfusion

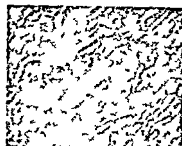


Fig 4 Dog No 833 Table 2 Embolic material in kidney No antifoam in circuit



Fig 5 Dog No 1080 Table 2 Embolic material in kidney Antifoam used

No definite identification of the embolic material has been made as yet It bears a resemblance to the material found in the oxygenator and differs from the ordinary protein precipitate seen in vessels The finding of the purple gray material when stained with H & E must be interpreted as suggestive evidence rather than proof of fibrin embolization None of the surviving dogs revealed evidence of infarction when sacrificed after 24 hours

With the utilization of the pump oxygenator in patients with myocardial infarcts or failure for prolonged periods of time the question of embolization and its significance appears even more important

Table 4

3 patients on partial cardiopulmonary bypass							
PATIENT	AGE	SEX	DEATH POSTBYPASS	BYPASS TIME	FLOW ML /KG	PROTAMINE TITER	PLASMA HG
56 1011	55	M	10 mo	5 hr	10 1	58	352
57 320	59	M	36 hr	4¼ yr	13 3	70	130
57 539	54	M	10 min	6 hr	8 1	60	

Flow ml /kg —ml /min /kg body weight (total flow from 700 to 900 ml /min)
Protamine titers—end bypass protamine titers in $\mu g/ml$

Patient 56 1011 was perfused 17 months following a mitral valvulotomy because of increasing failure and poor response to medical treatment The patient expired 10 months postperfusion Autopsy failed to show any changes which could be attributed to the previous bypass

Patient 57 320 had a massive myocardial infarct and died 36 hours post bypass Microscopic examination of the brain revealed the presence of basophilic hyaline partial obstructive thrombi in different vessels of the brain Comparison with the material found in dogs shows a resemblance The postmortem examination however revealed old as well as recent infarcts in lungs and central nervous system Thus the genesis of the recent infarcts is somewhat debatable

Patient 57 539 who had a massive purulent pericarditis died immediately following cardiopulmonary bypass No infarcts were present No examination of the central nervous system of patients 56 1011 and 57 539 was performed

SUMMARY

Postmortem examination of animals sacrificed at different time intervals following total cardiopulmonary bypass showed mainly pulmonary changes in animals sacrificed within the first 24 hours The pulmonary changes consisted of patchy pulmonary collapse and peribronchial hemorrhage Since the left auricle was drained in all cases in which cardiac fibrillation was used left auricular distention and pulmonary backflow was not considered to be responsible for the pulmonary changes[4] No correlation between perfusion volume blood flow arterial blood pressure oxygen saturation protamine titers and pathologic changes was seen

None of the animals receiving respiratory support during perfusion showed the pulmonary changes described above The presence of embolic material

was demonstrated grossly and microscopically in several animals sacrificed within the first 24 hours The material resembled that found in the oxygenator The significance of the embolic material is not clear and deserves further investigation

No changes related to the previous cardiopulmonary bypass have been found in animals surviving more than 24 hours

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STUDY OF THE EFFECTS OF CONTROLLED CARDIAC OUTPUT IN THORACOTOMIZED DOGS*

ERIC C ELLIOT AND JOHN C CALLAGHAN

A plastic well or device was developed which when introduced into the right atrial appendage of the dog permitted passage of catheters into the cavae In addition blood syphoned into a reservoir via the caval catheters could be pumped back into the right atrium through this device thus providing a method of controlling the amount of blood returning to the heart and indirectly the cardiac output This procedure was employed to study the effects of varying the cardiac output using the lungs as an oxygenator and the heart as a pump also it was used to replace the heart lung machine for studying different perfusion rates which in a previous study [1] was found difficult to do

METHOD

Healthy mongrel dogs 13 to 23 kg anesthetized with sodium pentobarbital 30 mg /kg were secured to the operating table in the dorsal position An endotracheal tube was passed and the cuff inflated Positive pressure artificial respiration was used after the chest cavity was opened

The perfusion apparatus was set up as in Figure 1 Mayon tubing was used for the vertical reservoir (2 in I D) and the ¼ in I D connecting lines A ⅜ in I D latex tube was used in the sigmamotor pump The lines latex pump and the lower inch of the reservoir were filled with saline prior to per

* From the McEachern Cancer Research Laboratory and Department of Surgery University of Alberta Edmonton Alberta With the technical assistance of John Engelhardt Supported by the Infant Heart Trust Fund and the Canadian Life Insurance Medical Research Fund

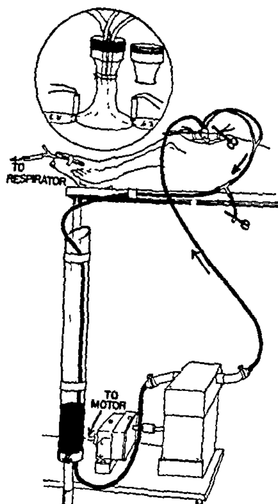


Fig 1 Schematic drawing illustrating the various components used for the perfusion

fusion and the pump roughly calibrated The lucite atrial cannulation device (see inset Fig 1), was 3/8 in I D at the tip This diameter admitted two 20 F catheters and left ample space around the catheters for the inflow of venous blood into the atrium An obturator to fit the device was necessary to introduce it into the atrial appendage

The right chest cavity was opened through the 4th interspace The cavae were circled with umbilical tape and firm rubber tubes placed over the tapes The azygos vein was ligated Heparin 2 mg /kg was then injected into the superior vena cava The pericardial sac was opened and an atraumatic clamp placed across the right atrial appendage The atrial device and obturator were introduced through an incision in the appendage and secured by a heavy suture just above the rim of the device After removal the obturator acted as a well through which the catheters were passed into the cavae The catheters, device and inlet line were filled with blood and connected as in Figure 1

At this juncture the control blood samples were taken from the femoral artery in 5 ml oiled syringes The control for the venous oxygen saturation determination was removed from the T connector just after commencement of the perfusion Blood pressure was determined by a mercury manometer connected to a needle in the femoral artery and the control BP was taken just prior to thoracotomy The perfusion period was set at 2 hours and the sigma motor pump was not altered during this period At the end of the 2 hours

the blood sampling was repeated and the flow rate of the sigmamotor pump accurately determined.

Oxygen saturations were determined by the Van Slyke apparatus The carbon dioxide contents were measured by the Kopp Natelson microgasometer and the samples were kept anaerobically. The pH values were determined semi anaerobically by the Photovolt meter. A correction factor of 0 011 per degree difference of temperature was used The pH and CO_2 determinations were done on the same sample of blood. The lactic acid was also determined

RESULTS

Five to ten minutes after the commencement of perfusion the blood in the reservoir rose to a constant level This initial volume of blood appeared to vary with the size of the animal and the flow rate. The results are presented in Figure 2 At low cardiac outputs a greater amount of reservoir blood was present in the group of larger animals, but as the flow rate increased this difference between the 2 groups decreased In general this initial reservoir volume of blood in the flow rate group of 20 to 30 ml /kg /min remained constant throughout the 2 hour perfusion Above this flow rate the level in the reservoir fell during the perfusion roughly in proportion to the blood loss from the increased oozing which occurred from the wound areas

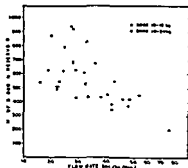


Fig 2 Represents the results of the relationship of the volume of blood that drained into the reservoir (as seen in Fig 1) to the cardiac output which was known by virtue of the fact that the only venous return to the heart (excluding the coronary sinus return which was not cannulated) was that allowed by the sigmamotor pump

Figure 3 presents the results of 15 dogs which were perfused for 2 hours and artificially respired with compressed air during this interval This group of animals invariably developed an alkalosis as evidenced by a comparison of the control and end perfusion values of CO_2 and expressed in mEq /L were respectively 16 5 (12 7 to 19 5) and 11 5 (6 7 to 15 5) Also, in this group of animals the arterial oxygen saturations were very poor in spite of what was considered to be adequate inflation of the lungs Average pre perfusion oxygen saturations were 81 3% From Figure 3 it would appear that the maximum changes in pH lactic acid and blood pressure occurred in the 20 to 30 ml /kg /min cardiac output group

After experimentation in thoracotomized dogs anesthetized with sodium pentobarbital, it was found that a mixture of 98% oxygen and 2% carbon dioxide would keep the pH and the CO_2 content of the arterial blood in good balance over a 2 hour period Figures 4 and 5 present the results of perfusions carried out in the same manner with this mixture The average preperfusion arterial oxygen saturation was 93 7% and the average end perfusion value 89 9% There was also a considerable improvement in the carbon dioxide values which preperfusion and end perfusion respectively were in mEq /L, 18 4 (16 1 to 21 7) and 16 6 (11 6 to 20 9) In Figure 5 the results of the

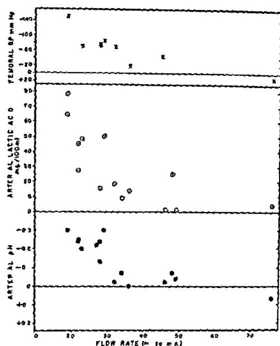


Fig 3 Results of 2 hour perfusions at different flow rates using compressed air to respire the dogs Results indicate the changes, either increase (plus sign) or decrease (negative sign) that occurred in the determinations during this interval

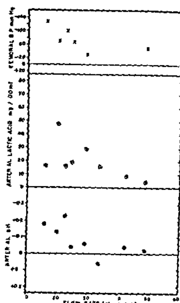


Fig 4 Results of 2 hour perfusions utilizing 98% O_2 and 2% CO_2 to respire the dogs

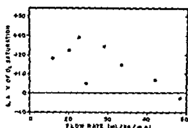


Fig 5 Presents changes of arteriovenous (A V) O_2 saturation difference during the 2 hour perfusion, respiring dogs with 98% O_2 and 2% CO_2 mixture

changes in A V O_2 saturation difference between the control value and the A V difference at the end of perfusion are graphically presented

By comparing the results in dogs breathing compressed air (Fig 3) with those breathing 98% O_2 and 2% CO_2 mixture (Fig 4) it is evident that the changes in pH and lactic acid were more marked in the former, but in both cases these occurred in the range of cardiac output 20 to 35 ml /kg /min

SUMMARY

A method has been described whereby different cardiac outputs in the dog may be easily created and the effects studied The procedure is analogous to experimental hemorrhagic shock except that the reservoir blood is recirculated and control of the cardiac output is obtainable Evidence is presented to indicate that the most marked changes in pH, lactic acid, blood pressure and A V O_2 saturation difference occur in the range of cardiac output 20 to 35 ml /kg /min It is felt that this method could offer potentialities for studying other effects that result from altering cardiac output

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EFFECTS OF VARIOUS PERFUSATES IN ISOLATED VASCULAR BEDS USING AN EXTRACORPOREAL CIRCUIT *

ROBERT J. RYAN, JAMES N. WINBLAD,
GEORGE C. HOTTINGER AND OSCAR CREECH, JR.

At the 1957 Surgical Forum a preliminary report was presented on perfusion of isolated vascular beds using chemotherapeutic agents for the treatment of malignant neoplasms.[1] Additional work has been reported elsewhere.[2, 3, 4] Since the original report additional animal experiments have been performed and over 50 patients have been treated using this technique. The most encouraging results have been noted using phenylalanine mustard in the treatment of malignant melanomas. The recommended doses of the various drugs are shown in Table 1.

Table 1. The Recommended Patient Dosage of Drugs for Perfusion through Common Femoral Artery

HN_2	0.8 mg./kg. B.W.
P.A.M. in Propylene Glycol	2.0
Actinomycin D	0.05
5 fluorouracil	20.0

Blood was used as the perfusate in all experiments and clinical cases previously reported. In addition to the usual disadvantages in the use of homologous blood it was found that nitrogen mustard is deactivated by blood. For these reasons an investigation of various blood substitutes for perfusion was undertaken. The use of different blood substitutes posed two major problems: first, the development of edema after operation and second, the effect of decreased oxygen tension on the activity of the chemotherapeutic agents.

Dabezies and Duke found the toxic endpoint of 5 fluorouracil in perfusion of the dog hind limb to be well defined at 20 mg./kg. of body weight. Therefore, blood substitutes were compared using this drug.

METHOD

Adult mongrel dogs were anesthetized with 30 mg./kg. pentobarbital. After exposure of the common femoral vessels heparin was administered intravenously in a dosage of 1.5 mg./kg. After cannulation of the vessels the previously placed tourniquet was tightened to occlude all collateral flow to the hind limb. Perfusion was then carried out for 30 minutes. Flow rates were recorded and the arterial pressure in the limb was monitored using a transducer and recording amplifier. The extracorporeal circuit consisted of a sigmamotor pump and a simple bubble oxygenator.[5]

* From the Department of Surgery, Tulane University School of Medicine. Supported in part by the National Cancer Institute, U.S. Public Health Service Grant #CY 3782 CY, The Anna Fuller Fund, and the American Cancer Society Institutional Grant.

Tissue oxygen tension was recorded using a bare tipped platinum electrode with a fixed potential of (—) 0 6 volts D C inserted into the calf muscle using standardized circuits ^

The perfusion of the limb with colloids (plasma and dextran) and the crystalloids (5% dextrose in water physiological saline Hartmann s solution and Darrow s solution) was compared with blood The various dosages of 5 fluorouracil was injected into the arterial line proximal to the pump head Following perfusion the limb was washed out using drug free perfusate

The extent of edema was determined by observation palpation and measurement of thigh circumference before and after perfusion Limb weights were recorded after sacrifice of the animal

RESULTS

To evaluate edema production by perfusion 7 hind limbs were perfu using blood alone Limb weights were normal at sacrifice up to 14 days foll ing operation No edema was noted in 6 limbs In high flow rates were use l
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previously emphasized

In contrast to blood the crystalloid solutions frequently produced edema ow rates has bee
This edema was unpredictable in severity and in its time of occurrence Sig nificant edema developed in 9 of 19 limbs perfused

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by oxygen 5 animals were vived To define the 20 mg /kg of 5 fluoroura genated blood All survived Five of 5 dogs also surv potentiation of 5 fluorouracil
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Table 2 The Relationship of Oxygen Perfusate and Dose of 5 fluorouracil on Survival of Dogs after Hind Limb Perfusion

PERFUSATE	DOSE MG /KG B W	SURVIVAL	TREATED
Nonoxygenated blood	25	5	5
Oxygenated dextran	25	5	5
Oxygenated dextran	20	6	6
Oxygenated plasma	20	5	6
Oxygenated blood	20	8	12
Oxygenated blood	25+	0	3

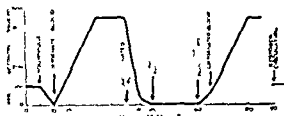


Fig 1 Graph showing the relative pO_2 in the calf muscle of the dog under the following conditions normal resting level complete vascular occlusion and during perfusion of the limb with oxygenated and nonoxygenated blood and blood substitutes

were perfused with nonoxygenated blood and 1 2 mg /kg of nitrogen mustard Both animals survived As reported previously [2] all dogs given more than 1 0 mg /kg nitrogen mustard in oxygenated blood failed to survive

The oxygen tension in the hind limb muscle during perfusion with crystalloid and colloid solutions (with and without oxygenation) was recorded and compared (Fig 1) The tissue oxygen tension was 35% above normal levels during perfusion with oxygenated blood The tissue oxygen tension fell to a constant level under conditions of total vascular occlusion and perfusion with nonoxygenated blood colloids or crystalloids Oxygenation of crystalloid solutions dextran and plasma increased tissue pO_2 only 2 or 3% above the pO_2 of total occlusion

DISCUSSION

The unpredictable appearance of edema after operation employing crystalloids as perfusates contraindicates their clinical use

The work of Gray [7] Churchill Davidson and associates [8] has shown that high oxygen tension potentiates the effect of radiation on tumor tissue Beck and Reick [9] demonstrated enhanced tumor susceptibility to radiation while the host animals breathed 100% oxygen Since most oncolytic agents have a radiomimetic effect potentiation of their action by oxygen should not be unexpected This study showed that oxygenation of the perfusates consistently enhanced drug toxicity The apparent potentiation of the action of chemotherapeutic agents by increased oxygen tension supports the continued use of oxgenated blood in cases of malignant neoplasms treated by isolation perfusion techniques

SUMMARY

This study shows that high oxygen tension increased the toxicity of 5 fluorouracil Dextran was found to be a satisfactory agent for washing out the limb at the completion of perfusion However dextran and plasma were unsatisfactory for perfusion with the cancerocidal drugs due to their inability to transport significant amounts of oxygen The unpredictable occurrence of edema following perfusion with crystalloid solutions rendered these agents unsatisfactory for clinical practice in the treatment of malignant tumors by the isolation perfusion technique

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THE EXPERIMENTAL AND CLINICAL USE OF A SMALL CAPACITY RAPIDLY AVAILABLE PUMP OXYGENATOR *

M MARTIN HALLEY DENNIS M L ROSENBERG ROBERT F RYAN
KEITH REEMTSMA AND OSCAR CREECH JR

One limiting factor to more diversified application of pump oxygenators has been the lack of a small capacity system which can be put into operation within a short time and with minimum advance preparation This report is concerned with a description of an extracorporeal circulatory unit that appears to meet these requirements

The unit consists of a plastic bubble oxygenator [1] together with the standard sigmamotor pump on a rolling table (Fig 1) The disposable oxygenator has a priming volume of about 500 ml and is capable of oxygenating up to 4000 ml blood per minute Priming may be done by withdrawing 500 cc of vena caval blood directly into the bag from the heparinized patient who is concomitantly transfused In emergency situations however banked blood (either typed and cross matched or type O Rh negative) wet plasma dextran or even saline may be used Venous blood is pumped into the oxygenator column and oxygenated then through a filter to the arterial reservoir Oxygen flow is maintained at minimum levels (0 5 to 1 L/min) to prevent foaming and bubble formation

To facilitate assembly the extracorporeal circuit is packaged and sterilized in three components (Fig 2) The oxygenator is sterilized by the manufacturer with ethylene oxide and is packaged ready for use The tubing for the pumps consists of latex rubber 3/4 in internal diameter with stainless steel adapters for 3/8 in tubing The final component consists of 10 ft of 3/8 in plastic tubing and a 3/16 in arterial cannula These sterile components can be quickly assembled and the entire unit may be left intact for prolonged periods if the tubing ends are sealed For emergency usages cannulation is

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* From the Department of Surgery Tulane University School of Medicine and The Tulane Surgical Service Charity Hospital New Orleans Supported in part by grants HTS5170 and H2628 National Institutes of Health U S Public Health Service

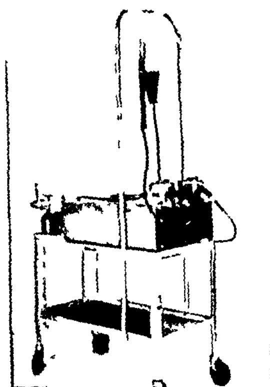


Fig. 1. The assembled pump oxygenator unit.



Fig. 2. The three component parts of the extracorporeal circuit are shown as they are packaged and sterilized for rapid assembly. (1) Latex rubber pump head tubing, stainless steel adapters, clamps. (2) Disposable plastic oxygenator. (3) Plastic tubing.

performed by inserting a single venous catheter through the femoral vein into the right atrium and placing the arterial inflow into the adjacent femoral artery. Cannulation and priming have been carried out within 5 minutes in the dog and 15 minutes in humans.

The unit has been used extensively in dogs for total and partial bypass procedures. Flow rates of 40 to 80 ml./kg./min. have been maintained in the total bypass procedures for periods up to 1 hour with survival.

Clinically, this apparatus has been used electively for perfusion of extremities, breasts, pelves, and lungs with chemotherapeutic agents[2,3] in approximately 50 patients without untoward results attributable to the extracorporeal circuit.

Emergency clinical perfusion has also been performed in two instances of cardiac arrest occurring in the accident room at Charity Hospital. One of these was secondary to gangrene of the entire small intestine and the other to hemorrhage from a ruptured spleen and lacerated liver. Cardiac massage for 60 and 40 minutes respectively was unsuccessful, but after support of the circulation was begun with the pump oxygenator system described above, vigorous cardiac action returned. Neither of these patients survived because of the extent of the primary injury. However, this experience demonstrated that the extracorporeal circuit could be put into effective operation within 30 minutes even under most unfavorable circumstances.

Further application[4,5] to selected clinical emergencies is planned, as it is believed that assisted circulation may prove of value in certain cases of cardiac arrest, pulmonary embolism, coronary occlusion, myocardial insufficiency, or pulmonary alveolar-capillary block, when rapidly available partial or total bypass may aid in resuscitation or may permit recovery by temporarily decreasing the work load of a failing myocardium.

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AN AUTOCLAVABLE STAINLESS STEEL MODIFICATION OF THE STATIONARY SCREEN OXYGENATOR*

JESSE E ADAMS EDWARD M LANCE
BAILEY F MOORE AND H WILLIAM SCOTT JR

With the acceptance of pump oxygenators as an adjuvant to clinical surgery it becomes desirable to define the requirements for a satisfactory unit for human application We have felt that the following criteria might be established (1) The system must be capable of delivering an adequate amount of oxygenated blood to any size patient (2) This flow must be obtained without undue trauma to the blood (3) The priming volume of the extracorporeal circuit must not be excessive (4) All component parts must be so designed as to permit complete and thorough mechanical cleaning (5) The system must be capable of being completely sterilized to insure the destruction of spores (6) The temperature of the blood passing through the system must be readily controllable (7) The materials and quality of construction must insure against unexpected mechanical failure

Eight months of experience with a vertical screen oxygenator (Mark G K)[1] † convinced us that although this particular unit is acceptable for clinical use the problems relating to its mechanical cleaning sterilization and temperature control necessitated a revision which would better satisfy the above criteria Adhering to the basic design of the Mark G K pump oxygenator we have designed and constructed a modification of this unit which has demonstrated its superiority in both experimental and clinical tests

METHOD

After evaluating available methods of sterilization steam sterilization was selected as the most desirable Thus it was necessary to utilize materials which

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† Manufactured by The Mark Company Randolph Massachusetts

* From the Department of Surgery Vanderbilt University School of Medicine Nashville
Supported in part by a grant from the Middle Tennessee Heart Association

withstand autoclaving without damage. Of the available heat resistant plastics and metals, stainless steel #301 was selected for the following reasons: 1) it is nontoxic; 2) it is sufficiently durable to tolerate repeated use; 3) custom designs can be fabricated from it without undue difficulty; 4) its surface can be polished to a mirror-like finish; 5) it is characterized by having a low electrical surface potential; and 6) it readily transmits heat.

The stainless steel unit was constructed so that it could be adapted to the same Mark G K frame, thus permitting the use of the DeBakey pumps for both the plexiglas and the steel systems.

One of the serious deficiencies of the plexiglas prototype was the difficulty in adequately cleaning it. This stemmed from the presence of inaccessible blind spots, internal threads which were difficult to clean, and seams and crevices in which debris and microorganisms could lodge. The new unit was designed so that it could be completely disassembled, thus eliminating all inaccessible areas. All crevices, seams, and sharp corners were eliminated and whenever it was essential to use threads, they were placed externally.

Although trauma to the blood has not been an objectionable feature of the Mark G K system, it was possible to further decrease this by several means. New connectors were constructed using a "knife edge" design which would not impede the flow of blood nor create unnecessary turbulence. The demonstrated tendency of the plastic tubing to kink was overcome by substitution of curved metal connectors and, in some instances, by realignment of the position of the connectors. A mirror-like #700 highly polished finish was obtained in all portions to further diminish trauma to blood.

The priming volume of the entire system was lowered slightly to 2800 cc by decreasing the size of all reservoirs. The addition of stopcocks to the regulating reservoir and to the bubble trap now permits blood to be added or withdrawn without exposure to air. An external circulating hot water bath about the arterial reservoir takes advantage of the thermal characteristic of stainless steel and permits absolute control of the patient's temperature during the bypass period.

To accompany the rigid portions of the system, it was necessary to have plastic tubing which could withstand autoclaving without loss of resiliency. Tygon tubing†† formulation S22-1, which we have previously employed, was not satisfactory after steam sterilization because of cloudiness and loss of resiliency. Acting on the suggestion of The United States Stoneware Company, Tygon tubing formulation B-44-3 was selected as it can be autoclaved without change of characteristics. This tubing has proven satisfactory in every respect.

After autoclaving, assembly of the component parts of the pump-oxygenator system is carried out on a sterile instrument table in the operating room just before it is to be used. The assembled unit is then transferred to the pump chassis and set in place, which obviates the difficulty of attempting to make sterile connections in an unsterile area.

The electrical control system of the pumps has been modified to offer greater insurance against unexpected failure. The control units themselves contain vacuum tubes which may cease functioning without warning. For this reason a third standby unit was added, together with a master control unit

†† Manufactured by The United States Stoneware Company, Akron, Ohio.

complete studies are available. In each instance, an accessory sucker system was employed to return coronary sinus or bronchial venous blood to the pump oxygenator.

Table 1

	BEFORE	AFTER
pH †	7 40	7 45
Platelets	116 000	66 000
PVC	37 8	36.5
Hgb*	12 1	12 1
RBC	4 37	4 11
WBC	5 850	4.500
Pl Hbg*	0 75 mg %	8.5 mg %

† Direct reading Beckman pH meter model H 2

Table 2

CASE NO	TOTAL AV FLOW (CC/MIN)	DURATION OF TOTAL BYPASS	PL. HGB INCREASE (MG %)	INDEX OF HEMOLYSIS
1	4800	41 min	4	2
2	5130	89 min	11	2 4
3	3190	36 min	19	16.5
4	3520	40 min	14	10
5	3130	40 min	6	4 3
6	3720	28 min	7	6 7
7	3800	48 min	9	4 9
8	4680	27 min	7	5.5
9	3800	30 min	6	5 3
10	4600	29 min	6	4.5
11	5000	57 min	7	2.5
12	4600	22 min	10	9 9
13	4050	40 min	7	4 3
14	2340	25 min	7	12
15	3850	49 min	21	11.2

SUMMARY

A stainless steel modification of the Mark G K pump oxygenator is described which has been successfully employed clinically This modification appears to offer the following advantages 1) mechanical cleansing is facilitated 2) Sterilization can be accomplished by autoclaving 3) Trauma to the blood is minimized 4) The temperature of the blood may be easily controlled

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ELECTRODE IDENTIFICATION OF THE CONDUCTION SYSTEM DURING OPEN HEART SURGERY *

JACKSON H STUCKEY BRIAN F HOFFMAN
PETER K KOTTMEIER AND HAROLD FISHBONE

Studies of conduction in the ventricles of the beating heart up to the present time have been carried out with surface and plunge electrodes The use of the plunge electrode is a relatively blind procedure and it has not been possible to chart conduction within the ventricle with the desired degree of exactness Recordings from the specialized conducting fibers have been obtained infrequently and by chance The development of the pump oxygenator and the technique of total perfusion now allow any chamber of the heart to be opened and studies to be carried out in a precise and unhurried manner It is the purpose of this paper to report a method for study of conduction within the heart under direct visual control

METHOD

Mongrel dogs weighing between 17 and 25 kg were anesthetized with sodium pentobarbital placed on controlled respiration, and the chest opened transversely in the fifth interspace

The left subclavian artery or one of the femoral arteries was isolated and prepared to accept the arterial cannula The azygos vein was ligated and a catheter introduced into the superior vena cava A second catheter was inserted directly into the inferior vena cava these two catheters returned the venous blood to the pump oxygenator [1]

The pericardium was opened and the external reference electrodes were sutured to the epicardium with fine arterial silk The injury current rapidly disappeared and was replaced by sharp complexes (Fig 1) The number and position of the reference electrodes are dictated by the problem being studied

* From the Departments of Surgery and Physiology State University of New York Downstate Medical Center Brooklyn N Y Supported by grants from the American Heart Association United States Public Health Service grants H 1250 and H 1011 and the Life Insurance Medical Research Fund With the technical assistance of Dr Antonio DeCarvalho from the Institute of Biophysics of the University of Brazil

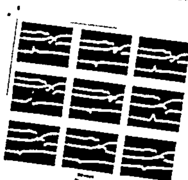


Fig 1 These tracings were obtained from the posterior aspect of the left ventricular septum at different levels In each of these readings the first and second lines are from reference electrodes placed on the right and left ventricular walls The third line is a tracing from the roving electrode

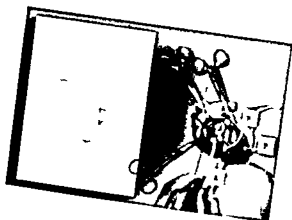


Fig 2 On the left of the figure is a blocked diagram as used in charting conduction within the right ventricle The X above the middle (anterior) papillary muscle is the point where the tip of the roving electrode (A) is placed for this particular reading The two reference electrodes (B) are sutured in place

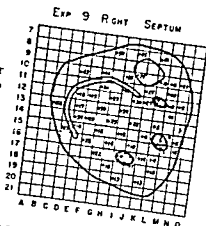


Fig 3 In the blocked diagrams of the right ventricular septum, the earliest (zero) point activated is adjacent to the superior aspect of the middle (anterior) papillary muscle The other numbers are milliseconds elapsing between activation of the points plotted and the zero point

The dog was heparinized and then placed on total bypass by tightening the slings placed around the superior and inferior vena cavae The right ventricle was then opened widely The traveling electrode was placed in predetermined areas (Fig 2) and tracings taken on a multibeam oscilloscope, these were projected onto 35 mm film and photographed at a speed of 250 mm /sec (Fig 1) The films were developed, read, and the data transferred to a blocked diagram (Fig 3) of the chamber of the heart under study Records were usually taken from 30 to 60 different locations within the right ventricular cavity

After charting of the right ventricle had been completed, the left ventricle was opened and recordings taken in the same manner The right and left auricles may also be studied as well as potential differences between the interior and exterior of the heart

The external reference electrodes consist of two fine silver wire contacts embedded in a lucite plaque The sutures holding these electrodes to the epicardium are 2 to 3 mm removed from the site of electrode contact In all experiments one contact of these electrodes is grounded and the other is connected to the input of the amplifier

The roving electrode is a curved plastic probe 10 cm in length It also contains two silver wire contacts separated by 0 5 mm and is used for bipolar recording at each point under study

Throughout the procedure the rectal and myocardial temperatures were recorded The temperature of the dog was controlled by using a Thermorite machine and by a heating element incorporated in the circuit A Sanborn Poly Viso was used for recording of the conventional electrocardiogram and the arterial blood pressure through a Statham strain gauge

RESULTS

In this pilot study of 10 dogs the earliest points activated in the right ventricular septum were anterior and were in the region of the origin of the papillary muscles In the majority of the hearts studied it was seen that the anterior middle and posterior septum were activated in that order In a minority this regular sequence was not observed As on the surface of the ventricles immediately adjacent points were not always activated sequentially Results of typical tracings are shown in Figure 1 In all experiments the earliest activity occurred at points where the specialized conducting system could be demonstrated either by electrical recording or by observation of hearts stained with Lugol s solution

In records obtained from the left side of the septum the electrical activity of the left bundle branch and ramifications was recorded at numerous different sites without difficulty This activity (Fig 1) appeared as a discrete deflection of short duration preceding depolarization of the myocardium by a variable interval On the right side of the septum the electrical activity of the specialized conducting system was regularly recorded only near the origin of the middle (anterior) papillary muscle Attempts to record from the common bundle and right bundle branch have not met with success up to the present time

SUMMARY

A method for studying conduction within all chambers of the heart has been described Advantages of this method are that records are made under direct visual control and all chambers may be studied minutely and unhurriedly With this technique the number of points that can be studied is not limited Records may be obtained from the ventricular myocardium and directly from the peripheral branches of the conduction system Further study will be required to determine whether or not this technique can be utilized both for purely investigative purposes and for the purpose of localization of the common bundle during repair of congenital defects

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Addendum The name of Dr Sandra I Saksena former Research Assistant should have been listed as one of the authors

AN EASY METHOD OF CONTINUOUSLY MEASURING FLOW RATES IN EXTRACORPOREAL CIRCULATION*

DAVID P. HALL, EDWIN L. BRACKNEY, ROBERT G. ELLISON

A simple accurate method of measuring flow rates during extracorporeal circulation is desirable. With a nonocclusive pump such as the DeBakey roller type pump, the flow rate for a given rpm will vary relative to the resistance offered by the arterial cannula and the vascular resistance of the patient. Two methods are described here by which actual flow rates can readily be determined regardless of the incompetency of the pump and the changing vascular resistance of the patient.

METHOD

In this method a modified Gibbon screen oxygenator purchased from the Mark Company with DeBakey roller type pumps was used. There are four variables which determine the stroke volume of one complete revolution: the height of the arterial reservoir, the volume of the tygon tubing within the roller runway, the degree of incompetency, and the resistance to flow offered by the arterial cannula and the patient. Since the level of the arterial reservoir varies only 2 to 3 cm, that factor can be discounted. Tygon tubing ½ in. I.D. and 11/32 in. O.D. with a wall thickness of 3/32 in. and 20 cm. in length was used always, thus making this variable a constant. The rollers were set to a maximum point of occlusion where they still turned satisfactorily. This distance had been measured between two arbitrary points and was kept constant for all pumping procedures. It will be shown later that at this distance the degree of incompetency remains constant over a wide range or resistance and flows, whereas when the roller is shortened only 0.3 mm. it is grossly incompetent and the stroke volume varies considerably. Therefore, with the roller at the proper setting, this variable remains constant for a given range of flows and resistances. This leaves only one variable to contend with, the resistance to flow as offered by the arterial cannula and by the patient. Perfusion pressure at any given pump speed is a function of this resistance. It is measured by inserting a mercury manometer (Fig. 1) to record perfusion pressure into the perfusion line distal to the arterial pump and proximal to the arterial cannula. This manometer is a simple U tube capable of measuring pressures up to 1000 mm. Hg. The zero mark of the manometer is at the level of the arterial pump. A 3/16 in. tygon tubing was connected to a T tube in the arterial perfusion line and connected to a sterile intravenous drip set that was connected to the mercury manometer. This gave an air blood interface in a cylinder with a volume of about 50 cc. The oscillations of the mercury column were damped by applying a screw clamp to the tubing containing blood. This in effect gives a mean pressure reading. Under these conditions flow rates have been determined for different settings on the rheostat control box against varying amounts of resistance as determined by the perfusion pressure. The flow rate was measured by direct flow into a graduate. The resistance was altered by a screw clamp on the arterial cannula with perfusion pressures varying between 20 mm. Hg and 600 mm. Hg. The resistance was varied also

* From the Department of Surgery, Medical College of Georgia, Augusta. Supported by research grants from the Georgia Heart Association and U. S. Public Health Grant H 2894.

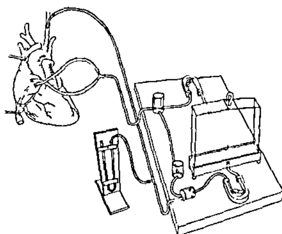
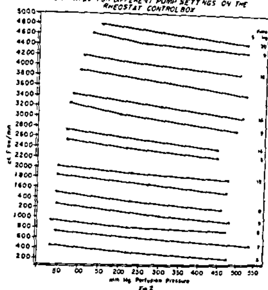
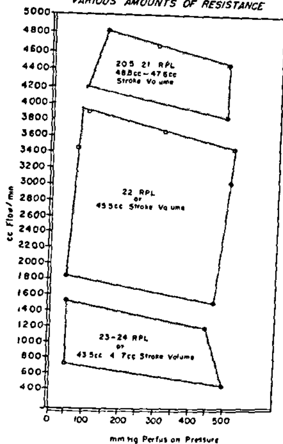
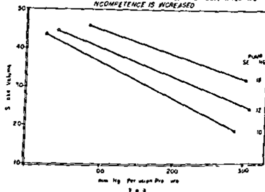
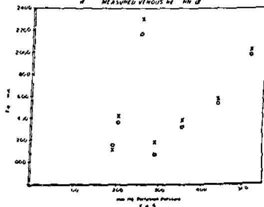


Fig 1









by two other methods which gave the same results The flow rates have been checked with both blood and tap water and gave the same results Therefore, the final calculations have been determined using the screw clamp for varying the resistance and water for the liquid for convenience sake As shown in Figure 2 one will note the flow rates that have been determined for a given setting of the rheostat at a resistance as reflected by the mercury manometer.

It is seen that the flow rate drops with increasing resistance at a given pump setting Thus the pump setting alone is not a true measure of flow rate However, the decrease in flow when the resistance increases is not due to an increase in the incompetency when the roller heads are almost occlusive but is due to a decrease in pump speed Because the setting on the rheostat control box is a gross estimate of the rpm it is felt that this offers a wide margin of error

With the use of a simple inexpensive revolution counter and stopwatch the rpm can be determined and if the stroke volume is known one can readily determine the flow rate Under the same conditions as previously described with the roller heads at the correct distance i e almost occlusive and using 20 cm of 1/4 in I D and 3/32 in wall thickness tygon tubing the stroke volume is determined in revolutions per liter As shown in Figure 3 the stroke volume of 15 5 cc or 22 rpl is constant within flow range of 1500 cc /min at 175 mm Hg pressure to 3900 cc /min at 100 mm Hg pressure This wide range will cover a great majority of desirable flows At lower flow rates the stroke volume is slightly less varying from 11 7 cc to 43 5 cc At higher flows the stroke volume varies between 17 6 cc and 48 8 cc The previously calculated stroke volumes can be rechecked before beginning a bypass by simply pumping into a graduated transfusion reservoir against resistance of a screw clamp and measuring the rpl It is felt that this method is probably more reliable than using the rheostat setting shown in Figure 2

In contrast to the nearly constant stroke volume with the roller set almost occlusive a slight change in the setting of the roller heads will greatly increase the incompetency of the pump as shown in Figure 4 In this figure the roller head was shortened 0 3 mm from the previous setting Whereas the rpl varied only from 21 to 23 a
varies between a low
simply knowing rpl
a true flow rate

Flow rates using the rheostat setting and perfusion pressure calibration method (Fig 2) have been checked on patients and dogs by measuring the venous return for a given time at a point when the venous return balanced the arterial outflow and there was no blood loss in the operative field These measured flow rates compared closely with those determined by the graph (Fig 5)

In addition to the determination of flow rate it is felt that the mercury manometer in the perfusion line also gives an added safety feature Any kink or obstruction about the arterial cannula is quickly reflected by a rapid rise in perfusion pressure and disastrous blowouts of connections can be avoided

SUMMARY

In summary a method of determining flow rates with a DeBakey roller nonocclusive pump is discussed If the pump is set almost occlusive and the same size and length of tygon tubing in the roller tract are constantly used then by measuring the rpm s one can obtain the flow from a previously determined stroke volume Also under these same conditions one can determine the flow from a previously calibrated graph by using the rheostat control box setting and the perfusion pressure

CEREBRAL ANOXIA RESULTING FROM HYPERVENTILATION*

WILLIAM MALETTI

In a previous report it has been shown that cerebral tissue anoxia may follow hyperventilation due to the accompanying hypocapnia or alkalosis[1] This paradox could be caused either by vasoconstriction or by alteration in oxy hemoglobin dissociation (the Bohr effect) that results from this alkalosis and hypocapnia The purpose of this study is to demonstrate that the cerebral anoxia which accompanies hyperventilation of the dog is due primarily to the Bohr effect and not to vasoconstriction

METHOD

Ten adult mongrel dogs anesthetized with 35 mg /kg body weight of intra venous sodium pentobarbital were used in this study Peripheral blood sam ples were obtained from indwelling femoral arterial and venous cannulae Venous blood from the brain was obtained by a Tocantin needle inserted directly into the sagittal sinus through a No 51 drill hole made through a short midline scalp incision In the dog such samples more specifically char acterize cerebral blood return than do jugular samples which include ample return from the rest of the head

Following a 45 minute stabilization period peripheral arterial, venous and sagittal sinus blood samples were obtained for determination of pH, nitrous oxide oxygen content, and carbon dioxide content

A control cerebral blood flow determination was made using the Scheinberg[2] modification of the Kety Schmidt[3] method with 15% nitrous oxide Simultaneously drawn blood samples from the femoral artery and sagittal sinus were analyzed on the Van Slyke apparatus for oxygen and carbon dioxide content

Hyperventilation was achieved by attaching a double lumen inspiratory expiratory endotracheal catheter to a mechanical respirator set to deliver 500 cc of 100% oxygen/kg of body weight per minute The degree of result ing alkalosis was monitored by serial 2 to 5 minute pH determinations on the femoral venous blood When the peak of alkalosis was reached (pH 7 6–7 7) repeat femoral arterial and sagittal sinus blood samples were drawn for oxygen content carbon dioxide content and nitrous oxide content

Cerebral blood flow was again determined immediately after these baseline determinations at the height of hyperventilation and alkalosis using an 85% oxygen—15% nitrous oxide gas mixture

Because of the large number of blood samples being drawn a 150 cc com patible blood transfusion was administered following the first cerebral blood flow determination to maintain normal blood volume

* From the Department of Surgery Veterans Administration Hospital Supported in part by U.S P H S Grant No A793(C3)
With the technical assistance of A Smith F Painter F Stoll and P Painter

Table I Results of Cerebral Blood Flow and Oxygen Utilization Under Basal and Hyperventilated Conditions

DOG NO	PEAK pH	TIME FOR HYPER-VENTILATION MINUTES	FEMORAL ARTERIAL CO_2 CONTENT VOL % CONTROL	HYPERVENTILATED	CONTROL	OXYGEN CONTENT VOL % CONTROLS ARTERIAL	VENOUS	DIFFERENCE	HYPERVENTILATED ARTERIAL	VENOUS	DIFFERENCE	CEREBRAL BLOOD FLOW cc/100 GM BRAIN/MINUTE CONTROL	HYPERVENTILATED	DIFFERENCE	CEREBRAL OXYGEN UTILIZATION cc/100 GM BRAIN/MINUTE CONTROL	HYPERVENTILATED	DIFFERENCE
II 13	7.55	00.45	12.15	39.80	—2.35	17.60	13.20	4.40	21.39	15.32	6.07	36.76	20.69	16.07	1.65	1.24	—0.41
II 14	7.66	00.10	31.26	28.72	—5.54	15.35	13.60	1.75	17.62	17.23	0.39	16.39	26.09	20.30	0.82	0.10	—0.72
II 15	7.68	00.22	15.09	33.72	—11.37	12.73	9.97	2.76	13.76	9.62	4.14	36.97	22.75	14.22	1.02	0.94	—0.08
II 16	7.67	00.37	16.82	27.60	—19.22	13.35	10.70	2.65	14.16	9.72	4.44	16.87	16.05	30.82	1.24	0.71	—0.53
II 17	7.69	00.30	34.99	27.67	—7.42	15.60	11.47	4.13	19.30	13.98	5.32	12.90	25.14	19.46	1.77	1.24	—0.53
II 18	7.65	00.22	50.61	30.31	—20.30	11.71	8.80	2.91	23.44	15.30	8.14	61.63	22.29	12.34	1.88	1.80	—0.08
II 21	7.62	00.23	36.39	19.41	—16.98	13.53	10.20	3.33	21.35	13.76	7.59	54.93	23.29	31.64	1.83	1.76	—0.07
II 22	7.61	00.48	50.77	32.28	—18.49	12.58	10.09	2.49	16.29	13.89	2.40	61.13	19.79	41.64	1.53	0.18	—1.05
II 23	7.79	00.19	19.01	25.69	—23.32	11.69	10.75	0.94	19.83	18.25	1.58	51.76	11.91	39.85	0.54	0.21	—0.30
II 24	7.67	00.31	14.55	32.00	—12.55	19.51	15.14	4.39	23.31	17.86	5.45	61.93	21.54	43.39	2.83	1.17	—1.68
MEAN	7.66	00.32															
DIFFERENCE					—13.71			AV 2.98			AV 4.55			AV 29.97			0.55
															t=3.13 P=<.01		

RESULTS

The results are summarized in Table I

The cerebral blood flow fell a mean value of 29 97 cc /100 gm brain/minute

The cerebral oxygen utilization fell in every case A *mean fall of 0 55* cc 0_2/100 gm of brain/minute These data have a P value of < 01 (Fig 1)

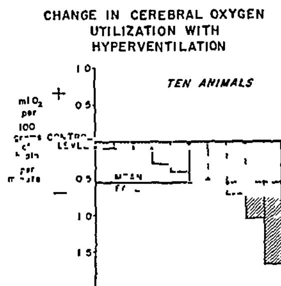


Fig 1 Cerebral oxygen utilization in control and hyperventilated animals

DISCUSSION

During metabolic studies on animals perfused by the pump oxygenator we[1] first noted the occasional bright red color of the venous return when the animals were hyperventilated by the oxygenator and were both hypocapnic and alkalotic The paradoxic high oxyhemoglobin level of the venous blood and simultaneous cerebral tissue anoxia were experimentally confirmed This seeming paradox could be caused either by the so called Bohr effect or by cerebral vasoconstriction that is known to accompany hypocapnia[4 5 6]

Bohr[7] in 1904 showed that alkaline hemoglobin resists the release of bound oxygen and others[8 9] have suggested that this property of hemoglobin might account for some of the signs of anoxia that may result from hyperventilation In our original report it was assumed that this was indeed the explanation for the proven production of cerebral anoxia following hyperventilation

Differentiation between that portion of cerebral tissue anoxia produced by diminution in cerebral blood flow from that portion due to the Bohr effect has been achieved by determining cerebral oxygen A V difference cerebral blood flow, and cerebral tissue oxygen utilization (cc /min /100 gm brain tissue)

If, on the one hand, vasoconstriction and lowered blood flow were the sole operative factor, cerebral blood flow would be diminished and oxygen A V difference would be increased as the cerebral tissue extracted more oxygen from the diminished volume of blood[10] These measured factors would result in no appreciable change in oxygen utilization (Fig 2)

If on the other hand, the Bohr effect were the only operative factor cerebral blood flow would remain unchanged but cerebral oxygen A V difference would approach zero since oxygen is poorly released from its hemoglobin

EXPECTED CHANGES DURING HYPERVENTILATION

	↓ FLOW	BOHR EFFECT	BOTH
A-V OXYGEN DIFFERENCE	↑	↓	↑ OR ↓
BLOOD FLOW	↓	→	↓
CEREBRAL OXYGEN UTILIZATION	→	↓	↓

Fig 2 Expected results of alkalosis and hypocapnia

bond under these conditions. This would result in a diminished tissue oxygen utilization (Fig 2)

Obviously both factors are operative under these experimental conditions since measured blood flow is diminished in all cases, but tissue oxygen utilization is also decreased The latter finding proves the Bohr effect plays an important role in producing cerebral anoxia during hyperventilation.

These findings have practical implications in the hyperventilation that occasionally is artificially produced during anesthesia, but more particularly the hypocapnia and alkalosis that can so easily result from improper monitoring of a subject on the pump oxygenator. It is apparent that hyperventilation and the resulting hypocapnia and alkalosis may produce tissue anoxia even in the presence of highly oxygenated blood. Monitoring of pump oxygenators should therefore include a pH or blood CO_2 analyzing device. Contrariwise, determination of the oxygen content of the venous return from the patient may give indirect evidence of the state of tissue oxygenation.

SUMMARY

1 By measuring cerebral blood flow and oxygen utilization in anesthetized dogs it has been shown that the cerebral tissue anoxia resulting from hyperventilation is due mainly to the Bohr effect and only in a small degree to cerebral vasoconstriction

2 The practical implications of this phenomena in the use of pump oxygenators have been discussed briefly

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CARDIOVASCULAR CHANGES ASSOCIATED WITH SELECTIVE BRAIN COOLING IN THE DOG *

C D Buster, Byron C Pevehouse, and H J McCorkle

In order to determine the effects of selective regional brain cooling on cardio-vascular function, a series of surgical physiological experiments was done with dogs A catheter was inserted into the carotid artery and arterial blood allowed to flow from it through a polyvinyl plastic tube, size 16 French, of sufficient length to yield a volume of 90 cc This tube was coiled on a frame in a cold water bath from which the blood was pumped (by a sigmamotor pump) back into the distal part of the same carotid artery and into the brain To rewarm the brain the water bath was heated slowly to 40°C A catheter was inserted via a femoral artery into the abdominal aorta and through it the blood pressure was continuously recorded from a Statham strain gauge connected through a direct current bridge balance to the recording oscilloscope and oscillograph Electrocardiographic recordings were made from leads applied to all four extremities The Brown thermocouple was used to measure the temperature of each cerebral hemisphere, the musculature of the right auricle and both ventricles of the heart, and the rectum Thermocouples were inserted into the cerebral hemispheres through surgical incisions which were then closed After placing thermocouples in the heart muscle through a thoracotomy incision the wound was repaired For controls, complete body hypothermia was induced by the ice bath method Because regional brain cooling induced general hypothermia to 30°C, a warming blanket was used when necessary to counteract this effect The animals were given 1 5 to 3 mg of heparin/kg of body weight intravenously to prevent clotting in the extra corporeal circulation Artificial respiration was used when the brain temperature was below 33°C Figure 1 illustrates the apparatus used to induce

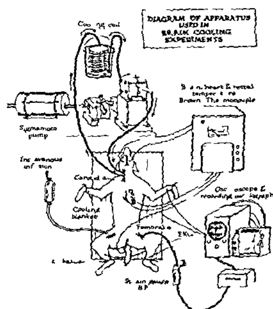


Fig 1 Diagram of apparatus used in brain cooling experiments (see text)

* From the Surgical Research Laboratories and the Department of Surgery of the University of California School of Medicine San Francisco Supported by the Christine Breon Fund for Medical Research

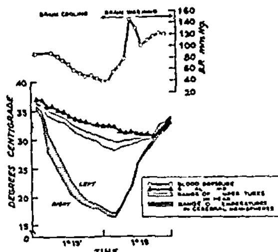


Fig 2 Graph showing typical response of the blood pressure to brain cooling Although the heart temperatures are somewhat lower than the rectal temperatures (during the cooling phase) they closely approximate the rectal temperatures Even though only the right common carotid artery was perfused the left cerebral hemisphere temperatures approximated the temperatures of the right cerebral hemispheres The blood pressure rapidly increased with brain warming and temporarily exceeded the original normal blood pressures

regional hypothermia of the brain and indicates the instruments used to measure the effects of brain cooling on the animal

Twenty five experiments were done The first few were observations of the blood pressure temperatures in various parts of the body, and electrocardiograph recordings during brain cooling Figure 2 is a graph of the records of these observations made during one experiment It is apparent that the heart temperatures are very nearly the same as the rectal temperature Also it is obvious that the (left) cerebral hemisphere contralateral to the carotid artery perfused with cooled blood changes temperature to almost the same degree as the other (right) cerebral hemisphere The blood pressure decreased to 40 mm Hg when the brain temperature was below 20°C, and then increased quickly as the brain was warmed This increase in blood pressure occurred rapidly before the heart and rectal temperatures began to increase Frequently as the brain was rewarmed the blood pressure was observed to increase above normal for a short period before returning to normal In the experimental animal extreme hypotension usually does not occur during general body hypothermia in the range of 28° to 30°C However in these experiments when the brain temperature was reduced to between 27° and 17°C the blood pressure began to diminish rapidly to measurements of 70 to 20 mm Hg even though the rectal temperatures were only 28° to 30°C (See Table 1) Figure 3 illustrates the data from an experiment showing a slight reduction in blood pressure which occurred during total body hypothermia induced by an ice bath but when hypothermia was induced by brain cooling while the heart and rectum were kept at near normal temperatures a marked hypotension occurred This hypotension disappeared when the brain was warmed

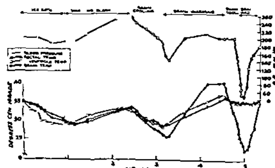


Fig 3 Graph showing that the blood pressure varied directly with the body temperature during general hypothermia but if the rectal and heart temperatures were kept normal while the temperature of the brain was lowered the blood pressure was lowered The blood pressure increased to normal values when the brain was rewarmed

Table 1 List of Rectal, Brain and Myocardial Temperatures at the Onset of the Lowest Blood Pressure Obtained during the Production of Regional Hypothermia of the Brain in 15 Experiments

DOG NO	BLOOD PRESSURE (MM HG)	BRAIN	TEMPERATURE (°C) RECTUM	HEART
111	20	15.5	31 1	–
90	30	23 0	30 0	–
159	20	19 0	31 8	29 0
16	30	15 0	32 5	–
198	30	14 0	33 0	–
19	70	15 9	30 0	29 2
30	30	18 8	34 4	31 0
76	45	22 1	35 2	33 1
77	35	18 4	31 6	–
225	40	21 7	31 2	29 8
13	35	16 9	27 7	26 2
52	65	24 2	21 6	29 7
212	55	16 2	34 4	27 4
53	30	16 8	30 2	–
79	40	17 0	32 3	30 0

Because of the possibility that extreme hypotension might be associated with myocardial anoxia, experiments were done to determine if the hypotension were reversible while the brain was still critically cool Figure 4 illustrates one of these experiments It shows how the blood pressure could be rapidly returned to normal with an intravenous infusion of norepinephrine or neosynephrine The amounts of the sympathomimetic drug used varied from 5 to 20 drops of a 2 mg 100 ml solution of norepinephrine or a 3 3 mg per 100 ml solution of neosynephrine per minute

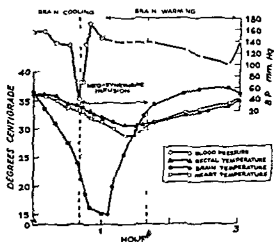


Fig 4 Graph showing the marked hypotension in an experiment of brain cooling reversed by a sympathomimetic drug while brain cooling continued

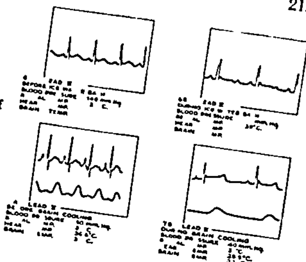


Fig [illegible] Diagrams of electrocardiograms
6A Before and 6B after induction of general body hypothermia
7A Before and 7B after brain cooling

Figure 5 shows examples of tracings of electrocardiograms (6A and 6B and 7A and 7B) made during these experiments These were (6A) diagram of electrocardiograph of an animal at normal body temperatures with normal blood pressures (6B) electrocardiograph of same animal cooled in an ice bath to rectal temperature 29°C the systolic blood pressure was 125 mm Hg (7A) diagram of electrocardiograph of another animal just prior to brain cooling with a rectal temperature of 37°C and a blood pressure of 150 mm Hg (7B) electrocardiograph of this same animal with a blood pressure of 40 mm Hg a brain temperature of 22°C a rectal temperature of 31°C and a right cardiac muscle temperature of 29 5°C These electrocardiographs made on experimental animals during brain cooling experiments show a broadening and slurring of the QRS complex with broadening of P and T waves and commensurate lengthening of the interval between the P wave and QRS complex and between the QRS and the T wave as the body temperature of the experimental animal became cooler They are similar to the electrocardiograph findings observed in experimental animals with general body hypothermia at temperatures of 28° to 32°C

SUMMARY

During the course of experiments in which selective regional hypothermia of the brain was induced it was observed that a marked reduction in the blood pressure occurred when the brain temperature was reduced to between 27° and 17°C The electrocardiograph records made during brain cooling were similar to those observed during general hypothermia The hypotension induced by cooling the brain was reversed by warming the brain or by giving sympathomimetic drugs while the brain was still cool

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THE INFLUENCE OF HYPOTHERMIA ON CEREBRAL INJURY RESULTING FROM CIRCULATORY OCCLUSION *

JACK M ZIMMERMAN AND FRANK C SPENCER

The effective use of the techniques of cardiac resuscitation has made it possible to restore cardiac action in patients who have had sufficiently long periods of cardiac arrest to produce severe central nervous system injury The central nervous system injury however has often resulted in death or severe neurological sequelae in patients who otherwise might have recovered from cardiac arrest One of the primary effects of the cerebral ischemia caused by cardiac arrest is cerebral edema If the cerebral edema could be prevented or decreased the neurological injury might also be decreased Hypothermia has been found to reduce cerebral edema and has been successfully used in 4 patients with severe cerebral injury following cardiac resuscitation [1] Because of the difficulty of obtaining control studies in patients the effect of hypothermia was experimentally evaluated by subjecting normothermic dogs to 10 minutes of cerebral ischemia and subsequently cooling the animals for 24 to 36 hours

METHOD

Twenty six mongrel dogs weighing 8 to 15 kg were anesthetized with endotracheal ether given with a mechanical respirator [2] The rectal temperature was constantly recorded and kept at 36 to 38°C A right thoracotomy was performed through the fourth intercostal space The azygos vein was ligated and the vena cavae and the ascending aorta isolated A 1 cm #15 gauge needle was placed in the ascending aorta and connected by polyvinyl tubing to a reservoir of heparinized saline suspended 100 to 130 cm above the aorta The administration of ether was stopped at this time The vena cavae were occluded and 10 to 15 seconds later the aorta was clamped distal to the needle Aortic occlusion was continued for 10 minutes during which time the cardiac action continued as the coronary blood flow recirculated through the heart and lungs The perfusion system maintained the aortic pressure at 80 to 100 mm Hg as higher pressures caused blood to flow into the reservoir and lower pressures caused saline to flow into the aorta

An alternative method of maintaining adequate aortic pressure was effectively used in 4 experiments Ten to 20 ml of saline were injected through a cannula in the right atrium whenever the aorta proximal to the occluding clamp became flaccid Although the intermittent injection of saline was a less sensitive method than the automatic reservoir perfusion it appeared equally effective and somewhat simpler to assemble After the 10 minute period of occlusion the occluding clamps were removed and the chest incision closed

The animals in the control group received no treatment except that they were turned every 1 to 3 hours while unconscious The treated dogs were immersed in cold water (8 to 10°C) immediately after the chest incision was closed and the temperature lowered to 31 to 33°C Hypothermia was maintained in this range for 18 to 36 hours Both groups of animals were carefully

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* From the Department of Surgery Johns Hopkins University School of Medicine Baltimore Supported in part by Grant H 2[illegible] National Heart Institute U S Public Health Service

observed for signs of neurological injury as shown by the level of consciousness respiratory activity pupillary reflexes and extensor tone and reflexes

RESULTS

All of the 26 animals subjected to 10 minutes of circulatory occlusion developed severe central nervous system injury as evidenced by coma lack of response to painful stimuli dilated pupils and slow, deep respirations No anesthesia was required for completion of the operative procedure

There were 12 animals in the control group (Table 1) Nine of these remained comatose and died in 8 to 30 hours Three of the 12 animals survived although 1 was blind The survival rate was 25%

Table 1 The Effect of Hypothermia on Cerebral Injury Resulting From 10 Minutes of Circulatory Occlusion

THERAPY	NO OF DOGS	NEUROLOGICAL RECOVERY RATE (PER CENT)	SURVIVAL RATE (PER CENT)
None	12	17	25
Hypothermia (31–33°C 24 48 hours)	14	79	57

Fourteen animals were treated with hypothermia Three of these remained comatose and died in a manner similar to those in the control group Eleven animals however showed a gradual recovery from the neurological injury over a period of 24 to 36 hours after which time they were conscious and could walk Three of the 11 however developed fatal respiratory complications in the following 36 to 72 hours The other 8 survived without detectable signs of neurological injury The over all survival rate was 57% but the recovery rate from neurological injury was 79%

DISCUSSION

These results indicate that hypothermia is of value in the treatment of cerebral injury from anoxia It was felt that the 79% recovery rate from neurological injury was a better measure of the effects of hypothermia than the 57% final survival rate because the deaths from pneumonia occurred after most of the signs of neurological injury had disappeared The pneumonia may have originated from pulmonary congestion and aspiration during the comatose period immediately following the cerebral injury Such deaths could probably be prevented by constant nursing care The fact that 21% of the treated animals did die from cerebral injury indicates that the benefit from hypothermia has definite limitations A limited study of greater degrees of hypothermia (26 to 28°C) did not show any increased benefit over that obtained at 31 to 33°C

It should be emphasized that the exact mechanism of the beneficial effects of hypothermia has not been demonstrated It may be that hypothermia decreases the amount of cerebral edema that results from cerebral injury from

anoxia If this explanation is correct hypothermia may be more beneficial if begun immediately after the cerebral injury rather than hours later when cerebral edema has already developed

The method of production of cerebral ischemia that was used in these experiments has not previously been described It was designed because the abundant arterial supply to the brain of the dog is difficult to occlude intermittently to a predictable degree and also because the circulatory interruption closely resembles that occurring in cardiac arrest The length of time that adequate cardiac function will continue when the aorta and cavae are clamped was not determined but the accumulation of metabolites would eventually cause cardiac arrest If the coronary venous blood were removed through a right atriotomy while the aorta was perfused with oxygenated blood cardiac action should continue for long intervals Such an experimental preparation can be easily used to evaluate the effect of any agent on cerebral injury from anoxia

SUMMARY

Severe central nervous system injury was produced in 26 dogs by occluding the ascending aorta for 10 minutes The survival rate in 12 animals receiving no further treatment was 25% In 14 dogs cooled to 31 to 33°C for 18 to 36 hours after the period of circulatory occlusion there was a 79% recovery rate from cerebral injury and an over all survival rate of 57% The incidence of neurological recovery was felt to be the more reliable indication of the effectiveness of hypothermia in diminishing the neurological injury following circulatory occlusion These findings confirm the impression gained from clinical experiences that hypothermia is of value in the treatment of cerebral injury following cardiac arrest

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Coronary Circulation

OBSERVATIONS ON MYOCARDIAL COLLATERAL CIRCULATION *

E A HUSNI AND F A SIMEONE

Coronary arterial occlusion is a well known effective stimulus for the development of intercoronary communications The contribution of these vessels to the circulation of myocardium made ischemic by occlusion of a branch of the left coronary artery has been the subject of extensive research in recent years Changes in the myocardial circulation in the area bordering on the ischemic myocardium (marginal zone) have received little attention In previous experiments it had been observed that when the descending branch of the left coronary artery was acutely occluded a visibly hyperemic area developed in the myocardium immediately adjacent to the well demarcated ischemic zone Objective data in relation to this hyperemic area would provide a better understanding of collateral circulation in the myocardium as well as elsewhere Therefore these experiments were undertaken to investigate some characteristics of this phenomenon by studying changes in the myocardial oxygen tension and temperature which would serve as indices of myocardial circulation

METHOD

Twenty two experiments were performed in which adult mongrel dogs were anesthetized with sodium pentobarbital (27 mg/kg of body weight intravenously) and placed on artificial respiration Records of the blood pressure were made on a direct writing oscillograph with a strain gauge connected to the left femoral artery A left thoracotomy was performed and the heart exposed by opening the pericardial sac ventral to the phrenic nerve A small segment of the anterior descending branch of the left coronary artery near its angle of descent was isolated over a thread in order to facilitate occlusion of the artery as needed The myocardial oxygen tension was measured by means of platinum microelectrodes These were made from platinum wire (diameter 0 075 mm) containing 15% iridium to which was soldered a very flexible teflon insulated copper wire to serve as a lead The platinum was insulated in capillary soft glass tubing and then coated with a thermosetting plastic compound † The tension of oxygen was recorded from the area directly tributary to the isolated artery (the central zone) from the adjacent areas of the myocardium (the marginal zone) and from normal myocardium (Fig 1) The temperature in the same areas of the myocardium was continuously recorded on the oscillograph by means of iron-constantan thermocouples

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† Plast sol Compound #2403 B G.S Plastics Co Cleveland Ohio

* From the Department of Surgery Western Reserve University and Cuyahoga County Hospital Cleveland Ohio Supported in part by Research Grant No 800 of the Cleveland Area Heart Society

imbeded beneath the epicardium in close proximity to the oxygen electrodes The pH of the myocardium in the same areas was determined by means of a glass hydrogen electrode (Beckman Model GS) Baseline values for the oxygen tension, temperature blood pressure and pH were obtained during a control period of 10 to 15 minutes Acute ischemia of part of the myocardium was then produced by occluding the isolated artery The occlusion was maintained for 2 to 5 minutes Records and readings were continued during this period of ischemia and during the 15 minute period after the arterial occlusion was released

RESULTS

The observed effects were similar in all 22 experiments with the exceptions noted below and were characteristic for each myocardial zone from which the data were obtained No change was noted in the normal myocardial zone in any of the experiments In the other two zones the following results were obtained

Central zone' A well demarcated area of cyanosis (Fig 1) was clearly recognized *within 30 seconds The oxygen tension and the temperature fell* sharply within seconds of the arterial occlusion in all animals (Fig 2) The greater part of this drop occurred during the first minute of ischemia and was more marked the further the electrodes and thermocouples were placed from the arborizations of the intact circumflex branch of the left coronary artery *The average drop in temperature* was 2°C *in 3 minutes* The tension of oxygen stabilized at a level of 10 to 40% of the control value The pH fell to a mean of 7 32 from a control value of 7 42 The blood pressure showed no significant change during this brief period of coronary arterial occlusion

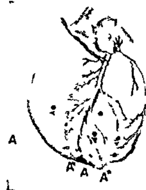


Fig 1 Diagram of the left ventricle (lower half of the diagram) with a thread around the anterior descending branch of the left coronary artery The shaded area (A) represents the zone made ischemic by occluding the illustrated branch of the coronary artery A represents myocardium with intact circulation and A (stippled) represents the marginal zone The solid dots represent the positions of the platinum electrodes and the solid triangles represent the positions of the iron constantan thermocouples

When the occlusion was *released* there was a visible hyperemia in the pre

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gradually returned to the control levels in 6 to 10 minute

to the control readings within 2 minutes

Marginal zone In this part of the myocardium the arterial occlusion led to a steady rise in the oxygen tension and in the temperature in 16 of the 22 animals (Figs 2 and 3)

The tension of oxygen attained a plateau 10 to 60% *higher than the control* level The rise in temperature varied between 0 25 and 1 0°C In the remaining *6 animals no significant change was observed* The pH of the myocardium in this zone remained unchanged Upon releasing the arterial occlusion all readings which had changed returned to the preocclusion values within 3 minutes

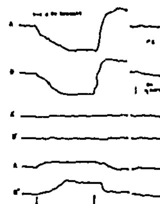


Fig 2 Oscillographic tracings illustrating the responses of the temperature and the myocardial oxygen tension in the ischemic zone (A B) in a normal area (A' B') and in the marginal zone (A" B") The first and second arrows represent respectively the start and end of the arterial occlusion



Fig 3 The effect of acute arterial occlusion and release upon the temperature and the tension of oxygen in the marginal zone of the myocardium The points represent means of values obtained in sixteen experiments The first and second arrows respectively represent the start and the end of the arterial occlusion

DISCUSSION

In contrast to the findings in the ischemic zone the myocardial oxygen tension and temperature in the marginal area rose steadily after the arterial occlusion and remained elevated throughout the duration of the occlusion (Fig 3) The rise in both the oxygen tension and the temperature can be accounted for only by increased blood flow provided by branches of the adjoining arteries This represents the initial phase in the formation of a collateral circulation Two mechanisms may be involved first ischemia and anoxia lead to the accumulation of acid metabolites as suggested in these experiments by a drop in the pH of the ischemic myocardium To be effective these metabolites must diffuse into the marginal area to act on the arterioles and capillaries there resulting in a visible vasodilatation over and above the resting circulation This observation is in keeping with the findings of Wang *et al* [1] who showed that in the anesthetized dog the flow of blood through the unoccluded arteries of the normal zone is substantially increased However there was no demonstrable change in the pH of the myocardium in the marginal zone such as might have resulted from the diffusion of metabolites into this area

The second mechanism to be considered is the fact that acute occlusion of an artery establishes a pressure gradient between the vascular bed of the occluded artery and that of the adjoining arteries [1, 2] This mechanism is of great importance for the development of a collateral circulation in response to rapid or slow arterial occlusions elsewhere in the body It has been observed in experimental animals that following an acute occlusion of the

femoral artery the peripheral pulse reappears within one minute[2,3] While this mechanism is effective in cases of occlusion of an artery such as the femoral, it can occur in the case of coronary arterial occlusion only in those animals in which the coronary arteries are not end arteries but communicate through functioning channels with the arborizations of neighboring coronary arterial branches Such communications have been anatomically demonstrated in only a small percentage of normal hearts[4,5] If the immediate hyperemia of the marginal zone does in fact depend upon the presence of these preformed intercoronary communications, the technique used in the experiments reported here demonstrates them in the majority of subjects (16 out of 22)

A mechanism such as the establishment of an increased pressure gradient could account for increased circulation through anastomoses between the coronary pericardial and the internal mammary artery However, occlusion of the left internal mammary artery had no effect upon the myocardial oxygen tension when tested in 4 animals

SUMMARY

Ischemia of a part of the myocardium was produced in the anesthetized dog by clamping the anterior descending branch of the left coronary artery The circulatory changes within the area tributary to the occluded artery and in the neighboring areas were evaluated by continuously measuring the oxygen tension, the temperature and the pH In the central part of the ischemic zone a marked drop was observed in the tension of oxygen, the temperature and the pH In the marginal zone of the ischemic myocardium however, the oxygen tension and the temperature showed a steady rise without a change in the pH Upon releasing the occluding clamp these phenomena were completely reversed The observations indicate that the changes associated with the formation of collateral circulation in the myocardium when a major artery is occluded are for the most part attributable to the establishment of an increased gradient of pressure between the intact arteries and the small intercoronary communicating vessels in the area immediately adjacent to the ischemic myocardium The data suggest that such communications are available in the majority of animals studied (16 out of 22)

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INTERCORONARY ANASTOMOSIS AND CIRCULATORY ADJUSTMENTS TO HYPOXEMIA INDUCED BY AN ARTERIOVENOUS FISTULA BETWEEN THE PULMONARY ARTERY AND LEFT ATRIUM *

Stacey B Day, Vincent L Gott,
John A Johnson and Owen H Wangensteen

Past surgical operations have attempted to augment the collateral circulation to the heart by the encouragement of vascularized adhesions by overlay cardiopexies utilizing various tissues and by procedures involving implantation of a systemic artery into the myocardium Few endeavors have been made to establish collateral growth by metabolic rather than by mechanical means Clearly this is the method by which the circulation to the ischemic myocardium is preferentially supplemented in clinical cases of impaired coronary artery distribution and would seem therefore to offer a good experimental channel for investigation Additional interest in this approach was stimulated by observations made on man at open heart surgery indicating the likelihood of rich intercoronary communications in patients with congenital cyanotic heart disease (Tetralogy of Fallot) The possibility that such anastomoses developed in response to cyanosis suggested the following experimental method

METHOD

Selected mongrel dogs 15 to 30 kg in wt were used All animals were anesthetized with 3% sodium nembutal (30 mg /kg body wt) and placed on a positive pressure respirator utilizing compressed air Left sided thoracotomy was performed in each case through the 5th intercostal space The pericardium was opened parallel to the phrenic nerve to display the main trunk of the pulmonary artery and the left auricle Using 5 0 silk a side to side arteriovenous anastomosis 10 to 12 mm in length was made in 10 animals between the pulmonary artery immediately distal to the semilunar valves and the left atrium Similar right to left anatomical shunts permitting a fraction of the blood to bypass the lungs were made in a further 10 dogs The studies in this group explored the effect of a fistula 17 to 22 mm in length After all operations the thoracic cavity was closed in layers and 500 000 units of penicillin given intramuscularly for 2 days At varying intervals (2 days to 14 weeks) following operation data on circulatory adjustments in response to the shunt were investigated Such information included observations on blood hematocrit hemoglobin pH and oxygen content Systemic blood for sampling of oxygen content and saturation was obtained from the aorta Venous sampling was from the coronary sinus Determinations were by the method of Van Slyke and Neill [1] In some cases pressure recordings were obtained from the pulmonary artery and left atrium and measurements of coronary sinus flow investigated Sufficient data are not available at this time to warrant precise comment

At autopsy sacrifice the pattern of the coronary arteries was studied by injection corrosion preparations [2] Previous studies of the architecture of the

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Supported in part by U S P H S —Cardiovascular Training Grant Anonymous Donor

coronary arteries in 50 normal dog hearts using the same injection method served as an adequate control study.

Observations presented relate the development of interarterial intercoronary anastomoses Data pertinent to the circulatory adjustments accompanying the production of these vessels are demonstrated in Tables 1, 2.

In those animals in which a fistula no larger than 10 to 12 mm in diameter was created there was no postoperative death. Exercise tolerance appeared to rapidly adjust and no dog appeared in acute respiratory distress At post mortem sacrifice the shunt was patent in all cases Of the 10 dogs with large anastomoses (17 to 22 mm.) 4 died within one month, although all survived 2 weeks postoperatively.

Of the survivors, 2 shunts were found thrombosed at autopsy. Study of the

Table 1. Circulatory Adjustments Induced by Arteriovenous Fistulae 10 to 12 mm. Length

DOG NO	WEEKS POSTOP	ARTERIAL O² CONTENT VOLS %	O² CAPACITY	O² SATURATION %	HBC CM %	HCT	DEGREE OF INCAPACITY
1	4	18 9	25 66	74 0	19 1	65	+
2	4	14 7	20 06	74 0	15 0	68	++
3	4	20 3	26 29	77 0	18 9	77	++
4	3	16 8	19 42	87 0	14.5	68	+
5	2	13 8	15 5	88	11 5	35	——
6	3	22 3	26 91	83	20 1	60	+

\+ Minimal Dyspnea
++ Dyspnea on Exertion

Table 2 A-V Difference· Values in Experimental Animals

DOG NO	pH	ARTERIAL O² CONTENT VOLS %	CORON SINUS O² CONTENT VOLS %	A V DIFFERENCE VOLS %
7	7 4	12 54	4 02	8 52
8	7 42	9 14	0 83	8 31
9	7 39	7 33	1 43	5 90

A-V Difference
Control Mean Values in 10 Experiments
Normal Dogs

ARTERIAL O² CONTENT VOLS %	CORON SINUS O² CONTENT VOLS %	A V DIFFERENCE VOLS %
20.5	5 1	15 4

animals in this group was confined to an examination of the coronary arteries at postmortem. Systemic arterial oxygen saturation in the reported animals varied from 60% to 88%.

Examination of vinyl plastic injection casts of the coronary arteries in the hearts of normal dogs demonstrated that intercoronary anastomoses were regularly to be found between the main rami of the left coronary artery. Communications of large arterial size were found in 30% of preparations. In no single case in this study however was an intercoronary anastomosis demonstrated between any branch of the right and left coronary artery in a control heart. Following the surgical production of an arteriovenous communication, 10 of 15 animals clearly demonstrated the existence of anastomosing channels between branches of the right and left coronary arteries in dogs sacrificed at intervals of 5 days to 4 months following operation. In every case (100%) richly abundant intercoronary networks were present between the major and minor rami of the left coronary artery. Of 5 hearts not injected, 2 were examined histologically, and 3 formed part of an acute circumflex artery ligation study to be reported.

Such objective demonstrations confirmed that not only did operation encourage the growth and development of new channels, (undoubtedly always present in potential) but with the union of right and left coronary arteries provoked an anastomotic bridge that served also to transport blood from the bed of one coronary artery to the bed of another coronary artery in which flow was presumably deficient.

Of considerable interest was the location and magnitude of speed with which these channels developed. The preferential sites for anastomosis between right and left coronary artery appeared to be on the dorsal surface of the heart in the anatomical region of the crux, or in the region of the apex mediated between a branch of the posterior descending artery and a branch of the right coronary vessel. Intercommunicating retinaculae appeared throughout the sternocostal surface of the heart. These channels, all arterial in size, were demonstrated as early as 5 days following operation. In several specimens within one month these vessels were of the same dimensions as large branches of the circumflex artery.

DISCUSSION

Such anatomical shunting clearly confirms the role of anoxemia as a powerful underlying stimulus for the development of interarterial intercoronary anastomoses. The circulatory adjustments to the hypoxemia related to this surgical procedure were characterised by a much greater arteriovenous oxygen *percentage* difference than that commonly occurring in normal controls. Simultaneously coincident polycythemia was a constant finding as indicated by an increase in hemoglobin and hematocrit values. The physiological trend appeared to be in a direction which would compensate for the lowered tension of oxygen in the circulating blood. Although only limited data are available, blood pressure did not appear to increase greatly in this form of hypoxemia, nor did pressure values in the left atrium differ markedly following the surgical creation of the shunt to those values recorded before operation.

The effects of anoxia may under some circumstances be stimulating. With respect to the heart, Hilton and Eicholtz[3] demonstrated an increased blood flow through the coronary vessels during anoxia. Subsequently Jochim[4]

showed that anoxia is a more powerful vasodilator of the coronary vessels than many preparations such as sodium nitrite and xanthine derivatives

It is noteworthy that in no revascularizing procedures examined in this laboratory aimed at creating intercoronary communications utilizing the same vinyl plastic injection mass has it been possible to demonstrate objectively intercoronary arterial anastomoses of the extent and dimensions observed in this study

The observations reported herein suggest that this method of creating interarterial anastomoses should be investigated in man with a view to its employment as a means of improving an impaired coronary artery inflow in patients suffering from myocardial ischemia The shunt could be closed by simple means it is felt after it had served the function of establishing adequate collateral intercoronary anastomoses

SUMMARY

Following the surgical production of an arteriovenous fistula between the main pulmonary artery and the left atrium interarterial intercoronary anastomoses between the right and left coronary arteries were demonstrated at postmortem examination in 10 of 15 dogs examined at intervals of 2 days to 4 months following surgery

The physiological trend of the circulatory adjustments to hypoxemia induced by this method appeared to be in a direction which would compensate for the lowered tension of oxygen in the circulating blood

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A BASIC OBSERVATION ON THE ABILITY OF NEWLY FORMED CAPILLARIES TO DEVELOP INTO COLLATERAL ARTERIES *

WILLIAM H. SEWELL AND DOUGLAS R. KOTH

Though much surgery for coronary arterial disease has been directed toward the redistribution of blood within the coronary vascular tree, an increased total volume is obviously desirable. This extra blood might theoretically be brought in by endarterectomy or grafting for removal of obstructions, anastomosis of the mammary artery to a coronary artery distal to an occlusion, or by a pedicle. The latter type of operation is most practical from a technical point of view, but though much work has been done, these procedures still have not achieved general acceptance.

Before transplanting more and different types of pedicles in dogs, it seems desirable to examine in detail the pertinent basic principles of collateral circulation formation to see whether these efforts are on a sound theoretical basis.

Study of the literature [1] reveals one common denominator found in situations where collateral arteries develop. These new channels will form between two arterial beds if, and only if, there is a difference in the pressure in each, and if there is also a vascular communication between them.

While the pressure in an artery in a pedicle might well be above that in a coronary artery distal to a block, the requirement of the vascular communication between the two must also be met. In most experiments in which these collateral channels were successfully produced, the initial communication was probably present since birth, and whether it was a capillary or a more highly differentiated structure is unknown.

The next question therefore is whether a recently formed capillary in the new tissue between the pedicle and the heart can function as the required communication, and develop into a collateral artery large enough to be of any value to the heart.

There is some evidence that it can. Plastic surgeons can construct a tube of skin and subcutaneous tissue which, after section of the second end, is nourished entirely by vessels which developed from recently formed capillaries.

Halsted in 1922,[2] while studying lymphatic drainage, and other workers later [3] transected the thigh of the dog except for the nerves, femoral artery and vein, and the bone. The divided tissue was sutured, and allowed to heal. If the femoral artery was divided within about two weeks of this operation, gangrene resulted. If it was divided at a later date, the limb survived. These latter legs were perfused by arteries crossing the previously divided site.

On the other hand, there must be a distinct limit to the size and number of collateral channels which can develop even under the most ideal circumstances. Otherwise, there would not be hypertension in patients with coarctation of the aorta.

The presently reported work was undertaken to find out the number and

* From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large

size of collateral channels which can form under the most favorable circumstances which might be hoped for with a pedicle and coronary arteries

METHOD

Surgery was performed on 6 adult mongrel dogs with strict aseptic technique One thigh was transected in the midportion, except for the femoral artery, femoral vein, femur, sciatic and saphenous nerves Hemostasis was obtained with 4 0 or 3 0 silk and the soft tissue accurately reapproximated with interrupted 3 0 silk sutures No antibiotics were given All dogs survived and walked on their involved legs without difficulty after about one week

The femoral artery was ligated and divided through a small incision at the level of the original wound in 5 of the dogs after 16 19, 18, 19, and 20 days respectively. The thigh transection operation was performed in the control sixth dog, but the femoral artery was not divided

No gangrene occurred Neither of the first two dogs walked on the involved legs after the second operation, but the other three walked without limp or other difficulty about 3 weeks after the second procedure

The first two dogs were sacrificed after 2 and 3 weeks respectively Mercury was allowed to run in through a cannula in the femoral artery placed near the level of the inguinal ligament, positioned so that the pressure at the line of the original soft tissue division was about 120 mm Hg X rays showed that both the proximal and the distal arterial trees were well filled with mercury, indicating that there was some arterial communication across the suture line but only several of these vessels could be traced on the film, and the diameter of the lumens was not more than 0 2 to 0 3 mm

In contrast, films made in a similar manner from legs of the other 3 dogs which were sacrified 9 weeks after the division of the femoral artery showed 10 to 25 definite connections across the suture line and the diameter of the lumens of these vessels was 0 5 to 0 7 mm These channels had the typical tortuous pseudo coiled appearance which we have learned from corrosion casts of hearts are characteristic of collateral arteries (Fig 1)

In none of the specimens was there filling of more than very small vessels in the marrow cavity The control dog had no mercury filled vessels crossing the line of transection

Attempts were made to study histologically the newly formed vessels It was impossible to prove that a given section was cut through the newly formed portion of the vessel However, some arteries were seen which had intima



Fig 1 The mid portion of the thigh showing mercury in the arteries The original site of transection of the soft tissue is transverse in this illustration and crosses near the center of the photograph It may be identified by the tortuous appearance of the collateral arteries

media, and adventitia, but were strikingly lacking in elastic staining elements characteristic of other vessels of comparable size, and these are believed to be the ones sought.

DISCUSSION

These larger vessels might have enlarged even more after a longer period of observation, but this seems unlikely because hemodynamic stability was probably reached during the 9 week period

Certainly a difference existed between the pressure in the arteries above the level of the femoral arterial division and that in the vessels below, and the only possible connections between these two beds from which the collateral arteries could have developed were the newly formed capillaries crossing the original line of division of the soft tissues

A single collateral vessel of the size observed would probably not be of significant value to an ischemic heart, but many of them would be, and there would seem to be no theoretical reason why these cannot be developed. Efforts will be directed toward the evaluation of basic factors in the construction of a pedicle to provide the most favorable conditions possible when a pedicle consisting of tissue with normotensive arteries is placed on a heart with experimental chronic coronary arterial hypotension

SUMMARY

Capillaries which formed across the suture line of partially amputated and reimplanted thighs of dogs showed themselves capable of development into grossly visible collateral arteries with lumens up to 0 7 mm after later division of the femoral artery at the same level These vessels connected the normotensive arterial system above to the initially hypotensive arterial system below

This observation implies that similar channels might theoretically develop from a pedicle containing normotensive arteries to hypotensive coronary arteries distal to a block in a region not already adequately perfused by collateral arteries

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CARDIOOMENTOPEXY AND IMPLANTATION OF MULTIPLE OMENTAL LOOPS FOR REVASCULARIZATION OF THE HEART *

FRANCES E KNOCK

To ameliorate the effects of multiple blocks in coronary arteries, omentum is being used to bring extracardiac blood to multiple deep and superficial areas of the heart In this laboratory, simple suture of omentum against the heart as advocated by O'Shaughnessy [1] produced relatively poor adhesions, even when used with talc and other irritants The O Shaughnessy procedure also allows revascularization of only superficial layers of the heart, although deeper layers may need added blood more than do superficial layers in patients suffering from coronary artery disease Consequently, in the present work epicardium is removed surgically or chemically to produce firm adhesions between omentum and superficial layers of the heart and multiple omental strips are implanted into left ventricle for revascularization of its deeper layers

METHOD

Figures 1 and 2 show the procedure used The pericardial sac is opened anterior to the phrenic nerve

incision in the anteromedial p

omental strips were implanted into the left ventricle and in 9 of these dogs, the left gastroepiploic artery was also implanted Thirty to 50 mg of talc were dusted over the surface of the left ventricle and the pericardial sac abraded Remaining omentum was then sutured against the left ventricle freed surgically of epicardium After 2 to 30 weeks, India ink and barium sulfate suspensions injected into the splenic artery below the diaphragm passed readily into the coronary arteries and emerged from the left coronary ostium Histologic studies revealed viability of the implants and the presence in each of one or more arteries large enough to carry barium sulfate Carbon particles were seen in capillaries between myocardial fibers in all of the hearts

In 10 other dogs, each with 8 to 17 omental strips implanted into the left ventricle and with epicardium removed only from the left ventricle, polyethylene sheeting and partially occluding heavy silk ties were wrapped about the origins of the circumflex artery and branches of the anterior descending artery to the left ventricle Clinically, all dogs remained vigorous After 1 to 3 months, wrapped arteries were found completely occluded grossly in 9 of 10 dogs Wrapped arteries in 6 control dogs were found to be only slightly occluded Complete occlusion of wrapped arteries at reoperation in controls caused prompt death Because the two series were not comparable, this method of testing was abandoned

In these omentopexy dogs whose coronary artery branches to the left ventricle were gradually occluded by polyethylene ties ligation of the omentum below the diaphragm 6 to 12 weeks after surgery caused no clinical or electro-

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* From the Department of Surgery, Presbyterian St Luke s Hospital and the University of Illinois College of Medicine Chicago Supported by a research grant from the Presbyterian St Luke s Hospital and by the Knock Research Foundation

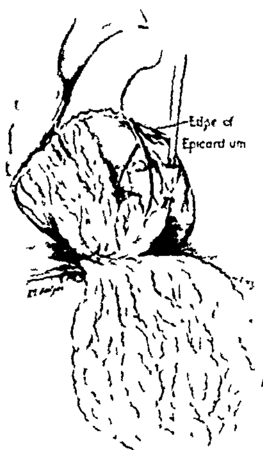


Fig 1 Method employed in implanting omental strips into the anterior surface of the left ventricle For complete cardio omentopexy epicardium is removed from both right and left ventricle as shown Omentum finally covers deepicardialized right and left ventricle and orgins of the great vessels and coronary arteries

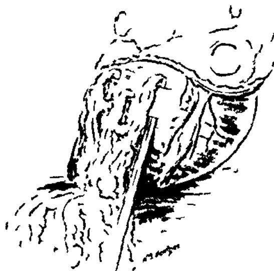


Fig 2 Method for implanting omental strips into the posterior surface of the left ventricle

cardiographic abnormalities At reoperation through the chest, however, with disruption of multiple adhesions of omentum to chest wall, lung and peri cardial sac ligation of the omentum close to the heart produced progressive electrocardiographic abnormalities and death on the table Thus omentum can parasitize the vascular supply of structures in the chest to bring extra cardiac blood to the heart

Extracardiac blood potentially available to the heart in these dogs with omentopexy only to the left ventricle, was estimated by heart lung by pass Inflow and outflow tracts were clamped, right ventricle and septum incised and blood collected from all chambers Control dogs gave steady state values of 0 3 and 0 1 cc /min operated dogs 4 6 and 7 7 cc /min The measurement involves disruption of a not inconsiderable proportion of the operation however because of the necessity of tearing down adhesions of omentum to chest wall and left lung to gain access to the hilum of the left lung

DISCUSSION

Because a substantial part of the value of cardioomentopexy lies potentially in the ability of the omentum to redistribute blood among various parts of the heart, efforts have been made recently to perform complete cardio-omentopexy encompassing both right and left sides of the heart For firm adhesion of omentum to right ventricle removal of epicardium from right ventricle is a prerequisite Surgical removal of epicardium is simple for left ventricle but difficult for right ventricle

Consequently, chemical methods for deepicardialization of the right ventricle have been investigated Phenol[2] has been used in patients for de epicardialization of left ventricle but can produce systemic toxicity In this laboratory, resorcinol, cresol, thiophenol, thiocresol, barbiturate salts acetic acid, lactic acid, phosphoric acid, ether, chloroform aliphatic alcohols salicylic acid, acetyl salicylic acid and salicylate salts have been tested for removing epicardium from the right ventricle Sodium salicylate solutions in hypertonic glucose have been found to be most useful Simple application of sponges soaked in 5% sodium salicylate solution to the right ventricle for a few minutes allows easy removal of long strips of epicardium from the right ventricle The chemical method also simplifies the surgical removal of epicardium from the right and left ventricle

At present, after removal of epicardium from the right and left ventricle and implantation of multiple omental strips into the left ventricle, a wide strip of omentum is laid over the origins of branches of the left coronary artery, over the conus arteriosus and onto the origin of branches of the right coronary artery, to supplement natural anastomoses between right and left coronary arteries in this area Another wide strip of omentum is laid over deepicardialized right ventricle If the omentum is short, the posterior two layers can be detached from the anterior two layers at the level of the transverse colon and unfolded to double the length of the omentum This more complete cardioomentopexy is now being challenged in dogs by use of Ameroid constrictors[3] at the origins of circumflex and anterior descending arteries

The major defect of the cardioomentopexy described is a low flow of blood to myocardium from each implant This defect can be remedied by use of multiple arterial implants from sources other than omentum At present multiple intestinal arteries are being stripped from small bowel and implanted into myocardium to supplement omentum as an extracardiac source of blood to the heart

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A COMPARISON OF VARIOUS TECHNIQUES FOR A SAFE AND RELIABLE METHOD OF CORONARY ARTERIOGRAPHY *

JACOB I. FABRIKANT, WILLIAM G. ANLYAN,
GEORGE J. BAYLIN, AND ROBERT B. TRUMBO

Aortography and selective peripheral arteriography have made possible the visual differentiation between diffuse and segmental arterial disease and have thereby permitted the selection of candidates for direct arterial operations such as bypass or replacement grafting or endarterectomy procedures. It is expected that the utilization of contrast material for the demonstration of the coronary circulation will 1) enable the surgeon to differentiate between diffuse and segmental coronary occlusive disease, 2) permit him to choose a particular palliative procedure to increase myocardial blood flow, 3) provide an objective criterion for the evaluation of existing surgical procedures, and 4) aid the surgeon in developing new operations to alleviate coronary insufficiency.

Unlike the extremity with obliterative arteriosclerotic disease, the heart is a highly irritable organ undergoing continuous rhythmic contractions. Arrythmias, fibrillation, and arrest are potential hazards of coronary arteriography. It is of extreme importance, therefore, to evaluate the variables of technique to provide maximum safety from permanent or even temporary damage and yet achieve adequate coronary visualization. This study was designed to evaluate systematically various techniques for a safe and reliable method of coronary arteriography in dogs with myocardial infarcts secondary to ligation of the left anterior descending coronary artery.

METHOD

Comprehensive studies were done on a colony of 16 previously healthy mongrel dogs weighing between 10 and 20 kg as follows: one week prior to ligation of the left anterior descending coronary artery, a baseline coronary arteriogram was done, followed by a second postligation arteriogram in 2 to 6 weeks. The dog was sacrificed and autopsied 48 hours later. The technique for arteriography considered most satisfactory has been described in a previous report by this group.[1]

Variables evaluated were as follows: A) for better contrast visualization, comparisons were made of 1) time and dosage factors in chemically induced cardiac arrest, 2) the filling of the arterial tree in the moving versus the arrested heart, 3) various radiopaque media in different concentrations, 4) the preferred location of the catheter tip, 5) hand injections versus automatic injector, 6) optimum pressure values for injection, 7) single tabletop films versus the Schonander Unit X-ray Machine, and 8) exposure time of the subject during the various procedures.

B) Methods for evaluating myocardial damage: comparisons were made of 1) EKG tracings taken before, during, and at periodic intervals after each procedure, 2) serum transaminase levels determined before and after each procedure, and 3) detailed histologic examinations of the heart carried out at

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Fig 5 A post ligation coronary arteriogram indicating the point of ligation of the left anterior descending coronary artery (arrow)



Fig 6 Pre and post ligation coronary arteriograms the latter 4 weeks following ligation of the lef[illegible] artery [illegible] through the posterior descending branch

the use of coronary arteriography in the human patient with minimum risk It is hoped that by the widespread practice of coronary arteriography in patients with clinical coronary arterial insufficiency, a true perspective of the pathologic anatomy will be derived This information may then be used to select, modify, and develop surgical procedures to alleviate coronary arterial insufficiency It is felt that arterial occlusion in the larger coronary vessels will be more frequently treated by direct operative means, for this reason the localization of these occlusions by arteriography will gain increasing significance The selection of candidates for direct coronary artery surgery versus the use of indirect palliative methods of increasing myocardial blood flow would be based on the interpretation of such arteriographic studies as advocated by Thal[3]

SUMMARY AND CONCLUSIONS

1 Various techniques of coronary arteriography were compared in a colony of 46 mongrel dogs

2 The injection of 30 cc of 50% hypaque with an automatic injector through an intraarterial catheter with the tip located at the junction of the brachiocephalic artery and the ascending aorta consistently produced adequate visualization of the coronary arterial tree

3 This technique was improved by the use of temporary acetylcholine cardiac arrest Acetylcholine cardioplegia provided a safe, transient asystole, reversion to normal occurred within 2 minutes

4 EKG monitoring serum transaminase determinations and histologic preparations detected no significant alterations following induced arrest and arteriography Because of its alleged capacity for secondary damaging effects the use of thorotrast should be restricted

5 There apparently was no added hazard to the use of coronary arteriography and acetylcholine arrest in dogs with myocardial infarcts Such experimentally induced lesions were depicted clearly

6 It is proposed that coronary arteriography may be employed as a special

diagnostic procedure in the evaluation of patients with coronary occlusive disease The selection of candidates for direct coronary artery surgery versus indirect methods of increasing myocardial blood flow may be based on such arteriographic studies

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A COMPARATIVE STUDY OF DIASTOLIC AND SYSTOLIC DYE INJECTION IN HUMAN CORONARY ARTERIOGRAPHY *

L. STEPHEN RICHARDS, RICHARD H GREENSPAN AND ALAN P THAL

The delivery of a rapid bolus of radiopaque media at the root of the aorta at the optimum time in the cardiac cycle has produced coronary arteriograms of sufficient detail to visualize the fine ramifications of the coronary system [1, 2] Objective evidence of coronary disease heretofore unobtainable has thus been made available and coronary arteriography assumes importance as a diagnostic tool and as an investigative aid in studying arteriosclerotic disease of the heart Knowledge of phasic filling of the coronary arteries goes back to Scaramucci [3] in 1689, who stated that the coronary arteries were emptied by the contraction of the heart and filled during its relaxation This report will present the techniques developed in this laboratory for phasic dye injection

METHOD

The injection device now used consists of two units, a nitrogen driven syringe and an electronic control system using the R wave of the electrocardiogram as a trigger Both components are mounted on a portable table providing a compact mobile unit The control system was specifically designed to allow injection of dye at variable times in the cardiac cycle and for any desired duration A photoelectric cell placed in the x ray field provided an impulse which marked the time of each exposure on the pressure and ECG tracing The record then indicated the length of injection and its precise stage in the cardiac cycle as well as the exact point of each x ray exposure (Fig 1) The catheter used was made of ordinary polyethylene tubing 2 92 mm in inside diameter and was selected because of the large lumen and relatively thin wall The tip of the catheter was heat sealed and three holes radially placed were made within 1 cm of the tip This arrangement diverts the dye laterally into the sinuses of Valsalva when the catheter is properly positioned Injection of dye through the aortic valve is thus prevented To make the catheter radio-

* From the Departments of Surgery and Radiology University of Minnesota Medical School Minneapolis Supported by U.S P H.S *Grant #3298* and a grant from Minnesota *Division of the* American Heart Association

paque and provide stability and a terminal curve tip for maneuvering, a stainless spring steel stylette was employed

Diatrizoic acid 90% was used initially as opaque media Recently 85% has been used because of a 25% reduction in viscosity as compared to a 5% reduction in contrast The lower concentration allows the delivery of a more rapid bolus at lower pressures

In order to evaluate the principle of phasic dye injection the following procedure was performed on 10 mongrel dogs under nembutal anesthesia An atrioventricular block was produced by placing a OOO silk stitch through the bundle of His [4] The cardiac rates produced by this method varied between 40 to 60 beats per minute The injection catheter was then positioned fluoroscopically 1 cm above the aortic valve plane A second catheter was placed in the aortic arch and a pressure tracing and ECG were recorded simultaneously on a two channel recorder From this record a test pattern for the actual injection was planned A proposed delay was measured using the R wave as the initial point Correct setting of the width circuit gave the desired length of injection

Four specific phases in the cardiac cycle were selected for evaluation systolic protodiastolic, middiastolic and end diastolic phases The duration of the delay was varied from 0 to 95 sec in order to inject in the respective phases The width or length of injection was held constant at 2 seconds under a pressure of 100 P S I and at 38 0°C allowing a delivery of 9 to 12 cc of 90% hypaque at each injection A series of 14 exposures at the rate of 5 per second were taken in the lateral plane to assess the rapidity and degree of coronary filling with the opaque material

RESULTS

The results are presented in Table I In all dogs examined the systolic injection gave the most complete coronary filling with the opaque medium (Fig 1 b and 2 b) It was noted that the progress of the dye along the major epicardial channels was rapid and exceeded the rate obtained when injection was made in the other stages of the cardiac cycle (Fig 1 a and 2 a) The opacity of the coronary arteries was maintained at a high level during several cardiac cycles With systolic injection the ejected blood from the ventricle is

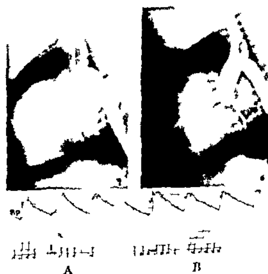


Fig 1 Dog 664— a) End diastolic injection X ray taken at conclusion of systole Poor coronary filling AP—Aortic pressure I injection Number on x ray at bottom of tracing indicates time of exposure b) Systolic injection—X ray taken at same time interval as in Fig 1a Note more rapid and opaque coronary filling

Table 1

PHASE	INJECTION: NO OF STUDIES	INJECTION: DURATION SEC	INJECTION: DELAY SEC	AMOUNT HYPAQUE DYE †	RESULT: CORONARY FILLING	RESULT: OPACITY	REMARKS
1 Systolic	6	2	0	9 11 cc	Excellent	Excellent	Rapid progress of dye in arteries Excellent opacity through several cardiac cycles Good filling of small peripheral branches
2 Protodiastolic	2	2	13	10 12 cc	Excellent	Good	Progress of dye in arteries less rapid Opacity diminished and remaining through 2 cardiac cycles
3 Middiastolic	4	2	5	10 12 cc	Good	Good	Progress of dye notably slowed Opacity not adequate but lasts 2 cardiac cycles
4 End Diastolic	7	2	75 95 ††	10 12 cc	Poor	Fair	Progress of dye slow Opacity very inferior No dye present in arteries after 1 cardiac cycle

† Hypaque 90% delivered through a polyethylene catheter (2 92 mm I D) under 100 P S I pressure at 38 0°C

†† This varied according to cardiac rate

mixed with the opaque medium At the conclusion of systole the bolus of dye remains at the base of the aorta where it is available to fill the coronary arteries The next ejection moves the dye from the major coronary branches into the fine ramifications where vessels 0 5 mm in diameter are visualized (Fig 2 b) Following this ejection the density of the opaque media remains excellent Ventricular contraction produces a marked tortuosity of the epicardial vessels *Engorgement of the coronary arteries is also noted* and is due to two factors first the transmission of the systolic pressure wave from the aorta second the volume elastic backflow from the intramural arteries to the epicardial arteries produced by myocardial contraction

The injection during protodiastole indicated only a minimal decrease in the rapidity of filling and opacity and in most respects was comparable to the systolic injection

The middiastolic injection produced only moderate coronary filling and opacity *for reasons which will be discussed in relation to the end diastolic* injection

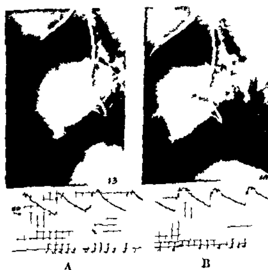
The least amount of coronary filling was obtained from the end diastolic injection *Progress of dye along the coronary arteries was considerably slower* than in the systolic injection The degree of opacity was definitely reduced particularly following the second cardiac systole (Fig 2 a) Comparing films secured at equal intervals following the systolic and end diastolic injections it appears that the initial phase of coronary filling with opaque material is considerably diminished in the end diastolic injection The ejection of blood from the ventricle following the conclusion of the injection moves the bolus of dye away from the coronary ostia into the descending aorta hence filling of *the coronary vessels with the opaque medium* is inadequate When the injection is made during late systole or early diastole the ejected blood is mixed with the opaque medium and remains stationary in the aortic bulb to supply the coronary arteries *throughout the whole of diastole (Fig 1 b)* This is particularly impressive when viewed under the fluoroscopic image intensifier

kely due

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to

coronary perfusion in the normal situation in large measure depends on the head of pressure in the aorta and that this perfusion continues at least to



A B

Fig 2 Dog 664 a) Same series as in Fig 1a Dye disappearing from coronary arteries Peripheral branches poorly filled b) Same series as in Fig 1b Systole has occurred Coronaries still well opacified Peripheral branches well filled



Fig 3 Human coronary arteriogram in 24 year old female with abnormal electrocardiogram but normal coronary system 35 cc of 90% Hypaque injected in 0 8 sec at a pressure of 150 PSI a) Film taken at conclusion of systole indicating poor coronary filling b) Film taken after 0.2 sec of diastole indicates rapid coronary filling

some extent during systole [5] The greatest surge of coronary filling occurs following the closure of the aortic valve when myocardial relaxation is complete and aortic pressure is still high

These findings have been confirmed in human patients It is demonstrated that during systole the coronary filling is not as rapid as in the early diastolic period Films secured at the beginning and at the conclusion of systole indicate standstill of the dye column in the coronary channels (Fig 3 a) The next film in the series taken during diastole demonstrates rapid progress of dye (Fig 3 b) For adequate visualization of the coronary arteries in man the injection is planned to begin in systole and end at some point in diastole depending upon time required to inject the necessary amount of dye This assures excellent coronary filling in all instances

CONCLUSION

The basic purpose of this study was to develop a method for delivering the least amount of dye in the optimal phase of the cardiac cycle that would give adequate and regular visualization of the coronary arteries With rapid injection of a small bolus of a radiopaque substance into the root of the aorta during the systolic phase of the heart dye dosage has been reduced to a minimum In our experience 30 to 45 cc of 90% diatrizoic acid depending on the weight of the patient will give excellent visualization of the coronary circulation in the adult human

SUMMARY

A brief description of the equipment and techniques in performing coronary arteriography is reported A comparative study of systolic and diastolic phase opaque dye injection is submitted and the efficacy of systolic injection is discussed A representative case demonstrating the degree of coronary artery visualization in the human with systolic injection is presented

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EFFECT OF CENTRAL DEPRESSION ON SURVIVAL FOLLOWING ACUTE CORONARY OCCLUSION *

F S HOFFMEISTER, W REGELSON, H RUBIN

Sudden death following acute coronary occlusion in man is frequently associated with the onset of ventricular fibrillation In dogs, acute circulatory failure develops concomitantly with ventricular fibrillation There is experimental evidence[1 2 3 4] (Table 1, 2) that the circulatory failure following myocardial infarction might be due to sensory stimuli carried via sympathetic afferents, superimposed upon myocardial damage

If this concept is correct, then the therapeutic attack in myocardial infarction might be directed toward the sympathetic block and/or central sedation rather than toward restoration of peripheral blood pressure

Table 1 Survival in Dogs Following Acute Coronary Occlusion

	NO	DEATH IN 1 HOUR	TOTAL % DEAD	TOTAL % SURVIVED
Conscious Control	12	8	66 6%	33 3%
Manning McEachern and Hall 1940—Low Tie	22	9	40%	60%

Table 2 Ligature Around Anterior Descending Ramus—One Stage Procedure Under Nembutal

	NO	DEATH IN 24 HOURS NO	%
Control	26	16	62%
Quinidine	26	12	46%
Staged Bilateral Thoracic Ganglionectomy	28	11	39%
Procaine Stellate Ganglion Injection Immediately Following Infarction	50	8	16%

Milch, et al Am Heart J, Vol 50, No 4 483–491 1955

* From the Departments of Surgery and Medicine Roswell Park Memorial Institute Buffalo New York Supported in part by funds from Smith Kline and French Research Foundation and Hoffman La Roche, Inc.

Experiments presented were designed to investigate the effect on survival following myocardial infarction of agents affecting sympathetic and/or sensory block. To this end a standard myocardial infarction was produced in dogs and survival under conditions of barbiturate anesthesia (pentobarbital) analgesia (morphine sulphate) and tranquilization (chlorpromazine reserpine) was determined. Survival of nonmedicated conscious subjects served as control.

METHOD

Mongrel dogs weighing 10 to 20 pounds were used. One week prior to the experiment proper under nembutal anesthesia a ligature was placed loosely around the anterior descending branch of the left coronary artery at its junction with the circumflex (See Fig 1). Loose ends were carried bilaterally through the chest wall and the chest closed. One week later infarction was produced by suddenly tightening the ligature. Two groups of animals were established: A conscious animals; B animals (1) under general anesthesia (pentobarbital) (2) under analgesia (morphine sulphate) (3) tranquilized animals (chlorpromazine reserpine). The following data were recorded: BP, EKG before during and after infarction, survival, gross and histopathologic picture after death or at sacrifice.

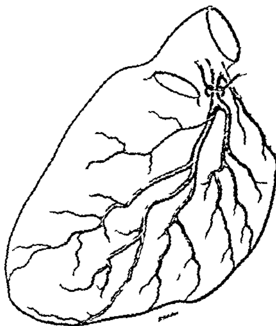


Fig 1

RESULTS

A. Survival of Conscious Animals. Within 5 minutes following occlusion ventricular fibrillation and death took place in 75% of the subjects (8/12) (See Table 3.) All animals which developed fibrillation died; all those in which

Table 3. Survival in Dogs Following Acute Coronary Occlusion of Anterior Descending Ramus of Left Coronary

	NO	DEATH IN 1 HOUR	DEATH IN 24 HOURS	TOTAL % DEAD	TOTAL % SURVIVED
Conscious Control	12	8	1	75%	25%
Reserpine 25 μg/kg i m 2½ 3 Hours Prior to Occlusion	10	2	1	30%	0%
Pentobarbital Anesthesia (Nembutal) 25 mg/kg	6	0	0	0%	100%
Morphine Sulphate 8 10 mg/kg i m 30 Prior to Occlusion	4	0	0	0%	100%

fibrillation did not develop survived No drop in BP was observed until the onset of ventricular fibrillation Figure 2 shows the correlation between the fibrillation and drop in the BP The range of onset of fibrillation was 1'10' to 10 10'

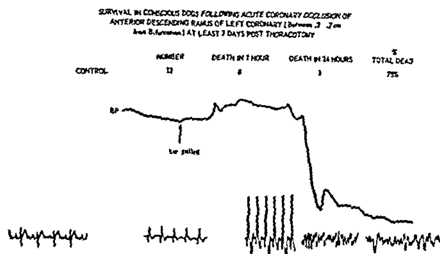


Fig 2

B Anesthetized Animals *Pentobarbital* None of the animals infarcted under pentobarbital anesthesia (25 mg /kg i v) died (0/4) (See Table 3)

Morphine Sulphate None of the animals given analgesic dose of morphine sulphate (8 to 10 mg /kg i m) 30 minutes prior to occlusion died (0/4) (See Table 3)

Chlorpromazine There was a difference between the effect of chlorpromazine given to conscious animals *which had just recovered from ether anesthesia* (given for femoral arterial catheterization) as compared with those in which *ether anesthesia did not precede chlorpromazine* administration

Chlorpromazine (2 mg /kg i v) following ether anesthesia death occurred in 28% of animals (2/7)

Chlorpromazine (2 mg /kg i v) without prior ether anesthesia but with procaine-ephedrine local anesthesia for femoral catheterization death occurred in 100% of animals (4/4) In animals given 10 mg /kg i v death occurred in 50% (1/2) (See Table 4)

Table 4 Survival in Dogs Following Acute Coronary Occlusion of Anterior Descending Ramus of Left Coronary

	NO	DEATH IN 1 HOUR	DEATH IN 24 HOURS	TOTAL % DEAD	TOTAL % SURVIVED
Conscious Control	12	8	1	75%	25%
Chlorpromazine 2 mg/kg i v Immediate Post Occlusion (30 45 Post Ether Anesthesia)	7	1	1	28.5%	71.5%
Chlorpromazine 2 mg/kg i v Immediate Post Occlusion (Procaine Ephedrine Local)	4	4	0	100%	0%
Chlorpromazine 10 mg/kg i v Immediate Post Occlusion	2	1	0	50%	50%

Reserpine Death occurred in 30% (3/10) of dogs given 25 μg /kg i m 150 minutes prior to occlusion (See Table 3)

CONCLUSIONS

The experiment indicates that, in dogs (1) shock is associated with ventricular fibrillation (2) ventricular fibrillation is invariably associated with fatal outcome (3) ventricular fibrillation is mediated via sensory and/or sympathetic pathways, (4) ventricular fibrillation can be prevented by a wide spectrum of nonspecific medication of sedative character It appears from the above that a therapeutic program for coronary occlusion in dogs might be effectively directed toward the sensory and/or sympathetic pathways

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THE USE OF AN INTRACARDIAC PACEMAKER IN THE CORRECTION OF TOTAL HEART BLOCK*

SEYMOUR FURMAN AND GEORGE ROBINSON

The knowledge that the mammalian heart either as a strip of muscle or as the intact organ can be stimulated to contract through the use of an exogenous electrical stimulus has been available for a considerable period of time However, it has been only in recent years that application of this knowledge has been extended to clinical medicine The earliest clinical use of exogenous cardiac stimulation was made by Zoll[1] in the application of electrical currents across the unopened chest to maintain cardiac rhythm in instances of cardiac arrest Since the start of cardiac surgery, cardiac arrest has become a common event postoperatively and A V dissociation with slow, idioventricular rate has been noted as a major cause of mortality following repair of congenital intracardiac malformations

Two major contributions to the concept of direct electrical stimulation of the heart have come from Weirich[2] and Folkmann and Watkins[3] Weirich with the implantation of a wire in the myocardium has been able to pace the ventricles from without and guide a patient with total heart block through the several postoperative weeks during which death would formerly have occurred Folkmann and Watkins were able through use of an ingenious amplifying device to stimulate the ventricles by amplifying the potential of the atrial systole and transmitting this amplified potential to the ventricles by means of two electrodes in contact with the ventricular myocardium

It has therefore been amply demonstrated that direct stimulation of the myocardium will produce safe and controllable contraction of the ventricles Zoll s technique however requires the use of high voltages across the closed

* From the Henry and Lucy Moses Research Laboratories the Surgical Division Montefiore Hospital New York City

chest, accompanied by convulsive contraction of the chest wall musculature. The methods of stimulation devised by Weirich and Folkmann require exposure of the heart by thoracotomy.

The situations in which prolonged electrical stimulation of the heart would be of advantage are many, ranging from permanent A-V dissociation with Adam-Stokes syndrome to sudden cardiac arrest in the operating room and major cardiac arrhythmias during diagnostic cardiac procedures. In each of these instances external stimulation may be undesirable and thoracotomy is best avoided.

The only portion of the heart available without resort to thoracotomy is the endocardial surface which can be approached with a cardiac catheter. We have found that stimulation of the canine heart can be undertaken as easily from the endocardial surface using an electrode introduced with a cardiac catheter as it can be from the myocardium directly.

METHOD

Sixteen mongrel dogs weighing 7 kg. to 20 kg. were operated upon, using intravenous nembutal or pentothal anesthesia. Total heart block was created using the method described by Starzyl.[4, 5] Right atriotomy was performed at body temperature, utilizing inflow occlusion. Upon exposure of the interior of the right atrium, a single suture, deeply placed at right angles to the annulus of the tricuspid valve and one centimeter anterior to the coronary sinus ostium will transfix and permanently destroy the conduction bundle.

A #8 Cournand cardiac catheter was prepared in the following manner. A stainless steel wire was introduced into its lumen, one end soldered to the catheter's metal shank and the distal portion of the wire soldered to a piece of metallic foil wrapped about the catheter tip to act as a conducting surface.

Upon electrocardiographic verification of the heart block, a single electrode was attached to the subcutaneous tissue of the thorax. The catheter was then introduced through the right external jugular vein and advanced until it lay in the right ventricle with its tip in contact with the right ventricular endocardium Both the subcutaneous electrode and the catheter, modified as an endocardial electrode, were then attached to the terminals of a pacemaker delivering pulses 0 to 3 volts D.C., 2 to 3 msec. in duration, monophasic and rounded and having a rate variable between 30 and 180/min. In several of the animals an additional electrode was implanted into the myocardium of the right ventricle and stimulation of the ventricle was undertaken alternately from the endocardial and myocardial electrodes

The right femoral artery was cannulated in each of these animals in order to record the femoral pulse wave and the changes in blood pressure occurring with variation in rate. This was also found necessary as each beat originating in the pacemaker superimposed itself upon the electrocardiogram so that the ventricular rate could not accurately be determined from the electrocardiogram itself Thus, the simultaneous ECG and femoral pulse tracings allowed observation of the rate of impulse formation by the pacemaker and the number of beats actually produced in the heart itself.

RESULTS

In 7 of the 16 animals true A-V dissociation was not produced. These animals exhibited a variety of bizarre arrhythmias and form the basis of our

observations on animals that did not have a complete A V block. The remaining animals had true, total A-V dissociation verified electrocardiographically and these animals provided the data concerning stimulation in A-V dissociation

In those instances in which both endocardial and myocardial electrodes were inserted, the voltage necessary to produce myocardial contraction was identical whether stimulation occurred from the endocardial surface of the ventricle or from the myocardium itself The lowest effective was 0 5 volts and in several animals voltages up to 1 0 volts were necessary.

1 Provided the voltage applied to the ventricle was sufficiently high, slightly above the minimal voltage necessary to produce a beat, there was one to one correspondence between the pacemaker impulses and the ventricular rate throughout the entire 30 to 180/min range. At voltages minimally effective at slower rates, raising the rate to 150 or 180/min will require increase in the voltage supplied the myocardium in order for complete correspondence to occur Allowing the lower voltage to remain results in occasional skipping of external beats when the rate is increased If the voltage applied to the ventricle is too low for complete correspondence then there are runs of pacemaker beats and runs of spontaneous cardiac beats intruding themselves upon the rhythm A minimal voltage will cause pacemaker dominance at slow pacemaker rates while a slightly higher voltage is necessary for the pacemaker to be completely effective at higher rates

2 Where a transient A V dissociation was followed by varying degrees of A V block with final reversion to RSR, the pacemaker could be made to take precedence, provided the external rate was more rapid than the spontaneous ventricular rate *and* the voltage high enough In these instances the sinus rhythm was suppressed Where there was change from A V dissociation to a sinus rhythm the only change necessary in the pacemaker was increase in the voltage supplied the myocardium to produce control once again If a minimally effective voltage is used to drive the ventricle, then conversion of the dissociation to a sinus rhythm is noted by a sudden escape of the ventricle with increase in voltage necessary to resume control Even arrhythmias occurring during the transitional period are immediately converted to the pacemaker rate and rhythm by raising the voltage to adequate levels

3 Where A V dissociation was permanent, a dependency on the pacemaker occurred where it was used more than momentarily When the pacemaker impulses were suddenly terminated there was often a lapse (nine seconds, the longest recorded), before a spontaneous ventricular beat occurred In several instances a single beat followed 1 to 2 seconds after cessation of stimulation only to be followed than by a long pause (Fig 1)

4 In those animals with permanent A V dissociation the imposed rate took precedence and a rate slower than the idioventricular rate could be maintained through the use of the pacemaker

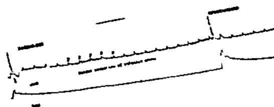


Fig 1 Dependence on the pacemaker with asystole following cessation of the stimulus

5 In rapid conversion from a slow (45 to 60/min) to a rapid (150 to 180/min) rate the ventricles had a period of 3 to 4 seconds during which complete correspondence did not occur During this time pacemaker impulses were followed irregularly, as if the ventricles required some time before they were capable of responding to the more rapid rate (Fig 2)

6 Approximately twice the voltage is necessary for stimulation if the positive rather than the negative terminal of the pacemaker is used as the endocardial electrode

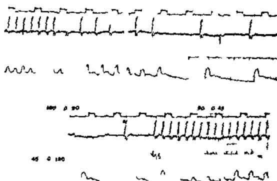


Fig 2 Response of the ventricles to sudden rate changes with maintenance of control

7 During runs stimulated via the endocardial catheter missed beats were rarely apparent even when definite A V dissociation was present It would seem that these instances occurred when the catheter lost contact with the right ventricular wall and momentarily floated free in the ventricular cavity This would seem to be in accordance with the x ray observation that there is contact between the catheter and the ventricular endocardial surface when stimulation occurs

8 Ventricular fibrillation is not affected by the endocardial pacemaker and voltages of extremely high levels applied directly to the endocardium have not had any effect in this series

9 Even following prolonged periods of stimulation 4 to 5 hours no evidence of trauma was found on the endocardial surface of the right ventricle

SUMMARY

A method has been described for electrical stimulation of the heart from the endocardial surface using an electrode introduced via a cardiac catheter The similarity to stimulation with a myocardial electrode has been stressed and the potential uses and advantages over other methods of electrical stimulation have been noted

Since submission of this paper for publication 2 patients with total A V dissociation have been maintained with use of the intracardiac pacemaker One during an operative procedure the second a patient with recurrent Stokes Adams attacks for the past 10 weeks with the catheter remaining in the heart for this entire period of time

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EVALUATION OF CARDIAC REVASCULARIZATION PROCEDURES USING MYOCARDIAL TEMPERATURE CHANGE DURING TEMPORARY CORONARY ARTERY OCCLUSION *

J. B. Brainard, C. M. Phibbs, and L. D. MacLean

The surgical treatment of coronary artery disease has been complicated by the lack of objective data supporting the many proposed myocardial revascularization procedures. Beck [1] has used the Mautz-Gregg backflow technique and comparative ligation of the anterior descending coronary artery to evaluate his procedure in dogs. Interpretation of the data from these techniques is rendered difficult, however, by the infinite anatomical variations of the coronary artery system of the dog. A method has been developed and is herein presented using a thermistor to evaluate the blood supply of the myocardium.

METHOD

A small thermistor † in the lumen, within 1 cm. of the tip of a 20-gauge needle was used to measure temperature changes while imbedded in the cardiac muscle. The thermistor itself consisted of a metallic oxide which offered resistance to a given electric current with temperature change. A constant current was applied across the thermistor and the resistance change with temperature was measured utilizing a Wheatstone bridge and galvanometer. The device was calibrated with a mercury thermometer so that the galvanometer could be read in degrees centigrade. Repeat calibrations at approximately 30 day intervals always checked within 0.02°C. over a range of 20°C. and individual readings could be made with an accuracy of 0.01°C.

Adult mongrel dogs were anesthetized with intravenous pentobarbital, intubated with a cuffed endotracheal tube, and respired with an intermittent positive pressure mechanical respirator using compressed air. The left chest was opened through the fifth interspace and the pericardial sac opened longitudinally. The anterior descending coronary artery was dissected out at the level of the root of the pulmonary artery and à closed right angle clamp passed behind it. Digital pressure on the artery could then be used to occlude it atraumatically against the clamp. The thermistor was placed in the myocardium between two branches of the anterior descending coronary artery distal to the place of occlusion (Fig. 1). A preocclusion temperature was taken, then the artery was occluded and readings taken at 30 second intervals. In 3 minutes the artery was released, and readings taken for 3 minutes more. The 3 minute period was chosen because it was long enough for a marked temperature drop to occur but not long enough to result in fibrillation.

During all experiments, simultaneous readings were taken with the myocardial thermistor and with a thermistor in a small polyethylene tube placed in the esophagus. All cardiac readings were corrected for total body temperature drift as measured by the esophageal thermistor.

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† Yellow Springs Instrument Co., Yellow Springs, Ohio

* From the Surgical Research Laboratory, Ancker Hospital, St. Paul, Minnesota and the Department of Surgery, University of Minnesota, Minneapolis. Supported by a grant from the Minnesota Heart Association

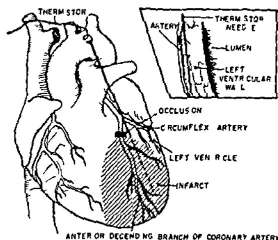


Fig 1 Placement of thermistor needle in the left ventricular myocardium

After a 3 minute occlusion a revascularization procedure was done using the tested area of the left ventricle for the anastomosis or implantation required by the procedure The chest was closed and the dogs were maintained in cages on a standard laboratory diet for 2 months A 3 minute occlusion of the same coronary artery as previously used was then carried out with the thermistor in the same area Exact repetition of the procedure was aided by a diagram of the coronary anatomy drawn for each dog on his record card at the first operation

After testing the animals were sacrificed Gross inspections of the hearts were carried out then they were either sectioned for microscopic study or the coronaries were injected with colored vinyl plastic* and digested in 10% potassium hydroxide to demonstrate anastomoses

To test the hypothesis that the temperature of the myocardium varied with the coronary blood flow a study was done in which the anterior descending coronary artery was gradually occluded with a Goldblatt clamp The cardiac temperature was recorded in the distribution area of the artery in the usual manner The temperature was found to drop when the flow was reduced by the clamp to remain stable at a given flow and to increase as the clamp was released

RESULTS

The data were recorded as an index of protection to allow convenient comparison among the various groups The index for each group was the difference between the temperature drop during the first and second occlusions expressed as a per cent of the drop during the first occlusion

Three general types of response to temporary coronary occlusion are apparent (Fig 2) The first type was shown by dogs which had undergone an implantation of the left internal mammary artery into the left ventricular myocardium (the Vineberg procedure)[3] When the implanted artery remained patent as it did in 54% of the dogs the temperature drop 2 months after operation was very small giving a high index of protection of 74%

The second type of response was shown after pericardial scarification and poudrage (the Beck I procedure)[1] cardiojejunopexy[4] cardiopneumopexy[5] internal mammary ligation[6] and pericardiotomy[7] The indices of protection for these procedures were similar ranging from 27 to 41%

The third type of response was found in a control group of animals tested

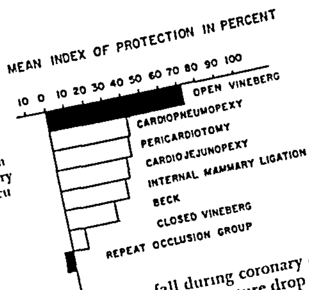


Fig. 2. Comparative protection against temperature decline during temporary coronary artery occlusion afforded by several revascularization procedures.

1 day after the initial determination of temperature fall during coronary occlusion. These animals showed essentially no change in temperature drop.

DISCUSSION

In this study, pericardiotomy represents the basic procedure done as a part of all other procedures. There is a statistically significant difference between the pericardiotomy group (10% protection) and the group in which occlusion was repeated within 1 day, indicating that the test procedure alone changed the blood supply to the myocardium. It is readily apparent that the groups of dogs undergoing pericardiotomy, cardiojejunopexy, and internal mammary ligation do not differ statistically in amount of protection obtained. Extracoronary anastomoses could be demonstrated in only 1 animal of these groups (a cardiojejunopexy), so the protection probably resulted from intercoronary anastomoses which were demonstrated. Comparison of the pericardiotomy group (pericardiotomy alone with retest in 2 months) with the Vineberg group (in which the implanted artery remained open) revealed a statistically significant difference ($T = 2.12$, $p = .031$). The open Vineberg group revealed a greater degree of protection. Since internal mammary implants with occlusions had only 9% protection, the mechanical trauma of implantation does not appear to cause more intercoronary anastomoses than the trauma of pericardiotomy. Thus it appears that the successful Vineberg procedure provides blood to the heart in addition to that supplied by intercoronary anastomoses. The difference between the pericardiotomy group and the groups which had occluded internal mammary implants or the Beck procedure was not significant.

SUMMARY

A method has been presented for measuring and comparing cardiac temperature drop with a thermistor imbedded in the myocardium before and 2 months after the Vineberg procedure, the Beck I procedure, cardiojejunopexy, cardiopneumopexy, internal mammary ligation and pericardiotomy.

The Vineberg procedure was found to bring a much greater increase in blood supply to a given area of normal dog myocardium than the other procedures tested. A substantial part of this blood came from extracoronary sources.

The test procedure alone or combined with any of the revascularization procedures gave a moderate amount of protection against temporary coronary occlusion, probably on the basis of intercoronary anastomoses.

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THE LYSIS OF ARTIFICIALLY INDUCED INTRAVASCULAR CLOTS IN MAN BY INTRAVENOUS INFUSIONS OF PURIFIED STREPTOKINASE *

ALAN J JOHNSON, W ROSS MCCARTY, AND WILLIAM S TILLETT

The fibrinolytic potential of streptokinase in the therapy of thromboembolic disease in man has been the subject of extensive investigation since its discovery in 1933[1] As a result of these studies *extravascular* fibrin clot lysis was produced in man[2] and *intravascular* clot lysis was produced in animals[3 4 5 6] but not in man

The present study was designed to demonstrate that 1) consistent and reproducible intravascular clot lysis can be produced in man 2) reformation of the clots can be prevented 3) the infusion of purified SK to activate the naturally occurring fibrinolytic enzyme of human plasma will bring about these objectives when SK is infused under defined biochemical conditions

Blood clots 5 to 20 cm long were produced artificially in the peripheral veins of volunteers Twenty four or 48 hours later SK was infused into a contralateral extremity The position and size of the clots were documented clinically and by venograms before during and after the infusions

Three methods were evaluated for their ability to produce clot lysis in vivo a) large amounts of circulating SK plasmin b) large amounts of free SK (or activator) c) small amounts of plasmin and free SK (or activator) †

A priming dose of SK was calculated to just neutralize the circulating antibody and inhibitor in each patient Therefore additional infused SK was free to produce an active fibrinolytic system

Subsequently a first sustaining dose of SK was infused in appropriate amounts for the production of method A B and C respectively

Finally the amount and duration of the second sustaining dose was

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† Since the assay used in this study does not distinguish between them the terms activator and free SK will be used synonymously

* From the Departments of Medicine and Surgery New York University College of Medicine and the Third Medical and Surgical Divisions of Bellevue Hospital New York Supported by grants from the Department of Health Welfare and Education (USPHS) and Lederle Laboratory Division American Cyanamid Company

determined by following the various biochemical constituents of the fibrinolytic system in the patient's blood, and by clinical and x ray appraisal of the experimentally induced clot

When clot lysis had occurred reformation of the clot was prevented in those patients with sufficient residual plasminogen by the infusion of additional SK

METHOD

Highly purified SK was diluted for infusion in 5% dextrose and ½% human serum albumen Since the amount of SK infused was extremely critical the rate of infusion was under constant surveillance

Occasionally a temperature elevation to 102°F or more occurred Amidopyrine was usually given during the ensuing 24 hour period ensuring its prompt return to normal

Thirty-eight blood clots 5 to 20 cm long were induced in the large superficial antecubital veins or the superficial saphenous veins of volunteers by direct irritation of the intima with a dental broach and chemical irritation with sodium morrhuate [7] A partial temporary impedence of the blood flow, by local pressure facilitated the prompt development of a fixed clot

No spontaneous lysis occurred in 13 controls The presence or absence of the clot was determined by inspection palpation and x ray venograms before during and after the SK infusions

Assays for SK and plasminogen were performed as previously described [3]

SK plasmin was determined by adding log dilutions of plasma to the standard clot system used above

When log dilutions of plasma were mixed with human plasminogen and added to the standard clot system above the additional fibrinolytic activity was thought to be caused by SK plasmin activator and/or free SK

Since Versene® † and other chelating agents commonly used to prevent the coagulation of blood also interfere with inhibition by the fast acting inhibitor (a) SK antibody and (b) SK plasmin inhibitor plus SK antibody, respectively were assayed by determining the reciprocal of the dilution of 1 ml of plasma and serum which would inhibit ½ of 1 unit of SK in the standard clot system These values were multiplied by 2 to correct for 1 unit of SK and were subsequently multiplied by the patient's estimated plasma volume to determine his circulating antibody and total inhibition

Fibrinogen was measured by the method of Blomback [7] and the prothrombin time was determined by the one stage method using Simplastin® †† and Acuplastin®, ††† respectively

In order to produce an active fibrinolytic system *in vivo* it was necessary to neutralize the patient's circulating antibody and inhibitor Thus the priming dose of SK was estimated by determining the amount of each prior to the infusion of SK The mean antibody for most of the patients included in this study was 191 977 units and the range was 10 250 to 470 000 The mean inhibitor was 175 046 units and the range was 43 000 to 410 000 Obviously, the range of both antibody and inhibitor was very wide and prior determination

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† Versene® brand of Disodium Ethylenediamine Tetraacetate Versenes Inc. Framington Mass

†† Simplastin® Warner Chilcott Laboratories Morris Plains N J

††† Acuplastin® brand of Thromboplastin Extract Orthopharmaceutical Corp Raritan N J

of these values therefore is essential in the estimation of an appropriate priming dose for each patient The total inhibition (antibody plus inhibitor) was thought to approximate the priming dose

The validity of this concept was shown by the fact that little or no SK plasmin was produced in the patient plasma until after the priming dose had been given and the further fact that plasminogen and prothrombin depletion did not start to occur until after the priming dose had been given These data were also confirmed by the unchanged fibrinogen and complement levels during this period

Method A Large amounts of SK plasmin were produced in this method After the priming dose had been given additional infused SK was free to produce an active fibrinolytic system Small intermittent doses of SK 25 000 to 50 000 units/day sufficed to produce large amounts of SK plasmin in the low antibody low inhibitor patients selected for this group The plasminogen was maintained at near normal levels in these patients in spite of the fact that the patient s own clotted whole blood lysed in less than 1 hour The prothrombin time was often markedly affected and in two instances went up to 50 seconds The fibrinogen was also affected by the SK plasmin and was virtually undetectible in the 2 patients mentioned above A few small areas of petechiae and ecchymosis also appeared on the surface of the skin in these (two) patients

Method B Large amounts of free SK were produced by this method After neutralization of the patient s circulating antibody and inhibitor a first sustaining dose of 100 000 to 300 000 units of SK was given This amount of SK usually sufficed to drive the plasminogen down from its normal level of approximately 5 000 units/ml of plasma to about 200 units/ml Little SK plasmin was produced by this method hence the one stage prothrombin time rarely rose to more than 23 to 24 seconds the fibrinogen was not markedly depleted and there was little danger to the patient

The continuous infusion of 45 000 to 60 000 units of SK per hour usually provided a considerable excess of SK Since the patient s plasminogen was already low the excess SK drove it down still further to levels of 50 to 100 units/ml of plasma and most of the clots lysed by this method reformed

Method C Small amounts of SK plasmin and free SK were produced by this method After administration of the priming dose and first sustaining dose of SK as in method B above the second sustaining dose of SK was infused at a rate calculated to maintain a small amount of SK plasmin and free SK This was usually obtained by infusing the SK at a rate of 25 000 to 35 000 units/hour Larger amounts of SK depleted the plasminogen excessively causing reformation of the clot Therefore appropriate adjustments of the infusion rate were necessary to maintain the plasminogen levels between 100 to 150 units until after the clot was lysed Frequent determinations of the patient s plasminogen plasmin free SK and prothrombin time assisted in maintaining this critical infusion rate

Clot Lysis *in Vivo* SK was infused in 7 instances according to method A (Table 1) Partial lysis was evident in 3 and complete lysis in 1 Circulating plasmin was present in all 7 without detectible free SK but no persistent clot lysis occurred SK was infused in 7 instances according to method B (Table 1) Two clots lysed and did not reform but complete or incomplete lysis was followed by clot reformation in 3 No lysis was detected in 2 Thus persistent

Table 1 Lysis of Intravascular Blood Clots in Man by the Intravenous Infusion of Streptokinase

Method of Therapy	No. of Clots	Clot Lysis Effected: Partial	Complete	Clot Reformed	Lysis Persisted	Total	No Lysis Effected
A	7	3	1	4	0	4	3 (1 48 hr)
B	7	3	2	3 (1 48 hr)	2	5	2 (2 48 hr)
C	11	0	11	0	11	11 (3 48 hr)	0
Total	25	6	14	7	13	20	5

lysis occurred in only 2 of the 7 treated by method B SK was infused in 11 instances according to method C (Table 1) Persistent clot lysis occurred in all Embolic complications were not observed with any of the methods

It may be noted in Table I that persistent lysis did not occur with 48 hour clots unless method C was used It was our impression that 48 hour clots took longer and were more difficult to lyse than the 24 hour clots

The course of a typical patient treated according to method C is shown by the x rays in Figures 1 and 2 One clot was induced 48 hours prior to the SK infusion (Fig 1b) and the other was induced 24 hours prior to the SK (Fig 2b) An infusion of 1 805 000 units of SK was given over a period of 36 hours The infusion was continued for 6 additional hours after clot lysis to prevent clot reformation The venograms in Figures 1c and 2c made 18 hours after the



Fig 1 Effect of infusion of streptokinase according to method C on intravascular clot induced 48 hours prior to infusion in patient G's right arm (a) right arm prior to induction of clot (b) 20 cm clot induced 48 hours previously shown immediately prior to SK infusion (c) persistent clot lysis as shown by venogram made 18 hours after SK infusion Lysis occurred in 30 hours Radiopaque lines at (1) and (2) define original area of clot induction Arrow at (3) indicates distal portion of clot



Fig 2 Effect of infusion of streptokinase according to method C on intravascular clot induced 24 hours prior to infusion in patient G's left arm (a) Left arm prior to induction of clot (b) 15 cm clot induced 24 hours previously shown immediately prior to SK infusion (c) persistent clot lysis as shown by venogram made 18 hours after SK infusion Lysis occurred in 18 hours Radiopaque lines at (1) and (2) define original area of clot induction Arrow at (3) indicates distal portion of clot

infusion indicate conclusively that both clots had lysed without clot reformation

DISCUSSION

The optimum biochemical conditions to effect consistent and reproducible clot lysis in man and prevent reformation of the clots obtain when SK is infused according to method C and small amounts of plasmin and free SK (or activator) are produced *in vivo*

Previous *in vitro* studies [8, 10] and *in vivo* studies in animals [3, 4, 11] have shown that fibrinolysis could be effected more readily by SK than by plasmin alone The present investigation has extended this observation to man if the infusion of SK is carefully controlled to prevent excessive depletion of the patient s plasminogen

The following hypothesis was formulated on the basis of these studies and the data presented above circulating plasminogen is adsorbed on the fibrin clot as it forms Adsorption effectively removes the plasminogen from association with and the effect of circulating inhibitors of both plasmin and activator SK is infused in amounts calculated to neutralize the circulating antibody and fast acting inhibitor Additional infused SK circulating free in the blood stream combines specifically with its substrate plasminogen which has been previously adsorbed on the clot Fibrinolysis is produced

The intimal damage to the vein wall persists Therefore there is a tendency for clot reformation to occur in varying degrees adsorbing both plasminogen and SK plasmin as it forms Consequently fibrinolysis recurs This sequence of events is thought to continue until intimal repair is effected preventing further reformation of the clot

Using this hypothesis method A was probably ineffective because the formed plasmin did not adsorb on the clot Furthermore a severe depletion of some coagulation constituents occurred with potential danger to the patient

Methods B and C were more effective because the SK was adsorbed on the clot The depletion of plasminogen in method B was excessive however preventing further lysis from occurring after the initial lytic phase Thus clot reformation tended to occur

Method C was obviously superior to the others presumably due to the adsorption of residual plasminogen on the reforming clot together with free SK and SK plasmin In this instance therefore the clot which reformed adsorbed all the constituents essential for its own destruction

SUMMARY AND CONCLUSIONS

Purified SK was infused in man to activate the naturally occurring fibrinolytic enzyme of human plasma

The optimal amount and duration of the SK infusions were calculated to effect sustained clot lysis SK was infused first to neutralize the patient s circulating antibody and inhibitor and second to produce a small amount of SK plasmin and free SK (or activator) in the circulating blood

These optimal biochemical conditions were maintained in 11 instances and persistent clot lysis occurred in all No embolic phe[illegible] were observed

The results demonstrate that SK will consiste[illegible] eproducibly lyse intravascular clots and pre[illegible]t reformation of th[illegible]n infused intravenously into patients [illegible] sely defined biocl[illegible]ditions

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EXPERIMENTAL STUDY OF THE ANATOMIC AND PHYSIOLOGIC EFFECTS OF INCISIONS INTO THE LEFT VENTRICLE *

W DEAN WARREN WILLIAM H MULLER JR MARSHALL EAST, AND OMAR SOSA

The operative mortality in closure of interventricular septal defects of the heart may be correlated directly with the degree of preexisting pulmonary hypertension In patients with essentially equal pulmonic and systemic arterial pressures and a small or absent left to right shunt, the risk of surgical repair has been prohibitive One of the questions raised by this experience concerns the ability of the myocardium to sustain a large wound and maintain an effective output against such resistance The aims of this study were to determine the response of the systemic ventricle to such a wound and to investigate the possibility of utilizing the left ventricle as an approach to intracardiac lesions in selected cases

METHOD

Mongrel dogs were anesthetized with intravenous nembutal and a preoperative electrocardiogram obtained Body temperature was reduced by external cooling to 31° to 34°C rectally and the chest was entered through the fifth left interspace By gently retracting the heart the superior and inferior vena cavae were visualized and a tape passed around them Quinidine gluconate (15 to 30 mg /kg) was administered to some animals as an antifibrillatory agent Pressure tracings were then recorded from the left auricle and left ventricle and a repeat electrocardiogram obtained at the lowered temperature During inflow occlusion an incision was made into the left ventricular cavity ranging between 5 and 8 cm in length The incision was

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* From the Department of Surgery University of Virginia School of Medicine Charlottesville Supported by U.S P H Grant No 2038

closed with a continuous OO silk suture and the average time of inflow occlusion was about 3 minutes An attempt was made to evacuate all air from the ventricle as the cardiotomy closure was approaching completion If ventricular fibrillation occurred an electric shock defibrillator was used in an attempt to restore normal rhythm Auricular and ventricular pressures as well as the electrocardiogram were recorded frequently during the early postoperative period A polyethylene catheter was positioned into either the left auricle or left ventricle and brought out through the chest wall for continued postoperative observations All animals were autopsied and those sacrificed were studied at a second thoracotomy just prior to death

RESULTS

Mortality Of the 20 dogs in the study 8 died during the operative procedure or on the day of the operation The commonest cause of death was primary ventricular fibrillation which occurred in 5 instances (this does not include the case of coronary air embolism) In this regard quinidine gluconate was found to diminish cardiac irritability and only 2 of 13 dogs receiving this drug succumbed to ventricular fibrillation On the other hand of 6 dogs given no quinidine prior to the cardiotomy 3 died of irreversible fibrillation However, 3 dogs in the quinidine gluconate group demonstrated excessive myocardial depression as evidenced by a marked bradycardia lowered arterial pressure and in 2 cases cardiac arrest One of these animals fibrillated following the administration of intraventricular epinephrine but this dog was the only one in the entire series to be successfully defibrillated Other cases of early death in this series were massive coronary air embolism postoperative pneumothorax and a large interventricular septal hematoma produced by an indwelling cardiac catheter

Of the remaining 12 dogs 5 died during the early days of the postoperative period 3 of these related to the ventriculotomy *per se* One dog failed to survive a prolonged hypotension which resulted from excessive blood loss during the cardiotomy Another had disruption of a portion of the cardiotomy closure and a fatal hemorrhage on the second postoperative day while the third died of brain damage from air embolism The other deaths were due to hemorrhage from the left auricle following dislodgment of the pressure cannula and dehiscence of the thoracotomy wound on the sixth postoperative day It is significant to note that in no instance was pulmonary edema or congestion found to be a factor except in those animals which had ventricular fibrillation or prolonged cardiac massage during the operative procedure

Pressure Recordings in the Left Auricle and Ventricle Pressures monitored in both the left auricle and left ventricle showed surprisingly little change in hearts that did not develop fibrillation or severe bradycardia In most animals there was some drop in the ventricular systolic pressure but it was felt that this was in part due to the loss of blood during the cardiotomy There was no end diastolic pressure increase in the ventricle save in those dogs with marked bradycardia following closure of the cardiotomy In 2 animals there was elevation of the mean auricular pressure and these dogs were found to have additional myocardial injury—one from air embolism and the other from a clot in the anterior descending coronary artery

In 2 animals sacrificed 14 months after left ventriculotomy there was a mild elevation of the left auricular pressure coinciding with the auricular

contraction on the electrocardiogram. These dogs were found to have some cicatrization of the anterior papillary muscle but with no evidence of mitral stenosis as determined by digital examination through the auricle in the living animal nor at autopsy. Consequently it was thought that this resulted from mild mitral insufficiency with increased filling of the left auricle. However, the auricular diastolic pressure was normal and there was no other evidence of cardiac failure.

Electrocardiographic Examination. In all animals there was very early evidence of ischemia as manifested by inversion of T waves. By the time the thoracotomy wound had been closed an injury pattern was present with marked ST elevation in the left ventricular precordial leads. In no case was there evidence of myocardial infarction except for the animals previously mentioned with embolization of the coronary arteries. Serial electrocardiographic examinations demonstrated definite improvement within a few days and in animals examined 10 days or longer after operation were essentially normal.

Anatomic. The gross and microscopic findings in this series correlated well with those previously reported by Thomas *et al.*[1] There was irregular broadening of the scar with rather marked thinning in some areas. The myocardium in these areas was replaced by dense collagenous tissue but in no instance was there evidence of aneurysmal formation. The endocardium healed more cleanly than the epicardial surface, often making identification of the internal scar difficult. There was no case of adherent thrombus to the site of the ventricular wound. There was invariably some degree of adhesive pericarditis over the cardiotomy site and in some instances the pericardium was quite thickened in this area. No instance of constrictive pericarditis was encountered, however.

Of special interest was the evidence of cicatrization of the anterior papillary muscle in the 2 dogs thought to have mitral insufficiency on the basis of their pressure tracings. In each instance there was thickening of the anterior papillary muscle and some chordae tendinae. However, there was no demonstrable hyperthrophy of either the left ventricle or left auricle in these cases and no damage to the valve leaflets themselves.

DISCUSSION

The techniques used in this study were adopted because of their simplicity and the minimal stress placed upon the myocardium other than that imposed by the ventriculotomy. One of the drawbacks of this procedure, however, is the increased incidence of ventricular fibrillation at lowered body temperatures. These findings confirm those of Stephenson and Main[7] that quinidine gluconate decreases the incidence of ventricular fibrillation in the hypothermic heart. Although in 3 dogs either a marked bradycardia or an actual cardiac arrest occurred with the use of quinidine, these hearts were resuscitated, whereas none of the animals in which quinidine was not used was successfully defibrillated.

These data indicate that normal myocardium is able to sustain a large wound and yet maintain an effective output against systemic resistance. From study of pressure curves there was often little evidence of impairment of ventricular function following such a procedure. There was usually a lowered systemic ventricular pressure following the cardiotomy for a brief period but in no instance was progressive ventricular failure seen to occur in those

animals not experiencing ventricular fibrillation or arrest at the time of operation

The anatomic findings were similar to those previously reported from the right ventricle[3] Although no aneurysmal formation was found in these animals this possibility cannot be ruled out from this study However, the relative narrowness of the band of myocardial loss, as well as the external support of the adherent pericardium would seem to give additional protection against this complication

Technical considerations in performing left ventriculotomy pose certain problems different from those in the conventional right sided approach The left sided approach necessary for adequate exposure of the left ventricle makes management of the vena cavae difficult and in the human would probably require a transsternal thoracotomy An additional anatomic consideration is the position of the anterior papillary muscle underlying the intercoronary groove to the left of the anterior descending coronary artery Although this is the optimal area for the cardiotomy from the standpoint of preserving the myocardial blood supply and gaining maximal exposure of the interventricular septum, the papillary muscle is so placed that incision in this area carries the risk of injury to this muscle or its chordae tendinae If the incision is placed far enough to the right to avoid injury to the papillary muscle, then branches of the anterior descending coronary artery system must be divided which imposes the added risk of myocardial ischemia However, incisions in the apical area and in the posterior region of the left ventricle near the right coronary artery may be performed without injury to the valvular mechanism or to major coronary arteries

A most important problem when opening the left heart is that of prevention of embolism Air embolism accounted for two of the deaths in this series and another dog had a myocardial infarction from a blood clot, apparently from the intraventricular catheter used for monitoring the ventricular pressure Very careful attempts must be made to evacuate all air and fill the ventricle completely prior to completing the ventricular closure

Finally, the problem of suturing the left ventricle requires special care From this experience as well as that of others,[4] it is thought that the full thickness of the heart wall should be included in a continuous silk suture The only instance of cardiac wound disruption occurred on the second day following a so-called epicardial closure

CONCLUSIONS

1 Normal dogs can survive large left ventricular incisions performed under hypothermia

2 In those dogs not surviving the procedure, the mechanism of death has not been a progressive left ventricular failure

3 Quinidine gluconate lowers the incidence of ventricular fibrillation but with increasing dosage undesirable myocardial depression may occur

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THE CARDIAC CONDUCTION TISSUE AND ITS BLOOD SUPPLY IN THE DOG*

GEORGE LUMB AND R S SHACKLETT

Examination of the atrioventricular node and the bundle of His and its branches in the dog reveals a morphological appearance and anatomical distribution which is similar to the human[1,2,3,4,5,6,7,8,9] In both animal species the conduction tissue of the atrioventricular node area and bundle of His is seen to be in direct relationship to a profuse capillary network As the canine cardiac blood supply is sufficiently similar to the human to make it a suitable experimental animal it is the purpose of this work to define the vessels supplying the blood to this capillary bed in the dog and to investigate the changes which occur when they are occluded

Anatomy Two principal vessels supply branches to the upper part of the interventricular septum and floor of the right atrium One is a posterior vessel which we call the posterior septal artery The other is an anterior vessel the anterior septal artery The posterior artery arises in all cases from the left circumflex artery at the point where the posterior descending branch courses down the middle of the posterior surface of the heart It passes anteriorly just to the left and inferior to the termination of the coronary sinus It gives branches to the walls of the left and right atrium and ends in capillaries near the root of the aorta It is from this vessel that the blood supply to the atrioventricular node and bundle originates Along its course it gives off arterial twigs which pass through the annulus fibrosus to end in the upper part of the interventricular septum Near its origin it frequently gives a branch to the upper part of the right ventricular wall which anastomoses with the first lateral division of the posterior descending artery In our experience on one occasion only communication has been demonstrated with terminal branches of the right coronary artery On three occasions two separate vessels have arisen close to each other in place of the single posterior septal artery

The anterior septal artery arises quite constantly from the posterior surface of the left coronary artery at the point of division of the anterior descending and circumflex branches or within 2 to 3 mm along the course of the anterior descending artery[10] It passes posteriorly in the posterior wall of the infundibulum of the right ventricle just inferior to the cusps of the pulmonic valve It enters the interventricular septum and divides to give one branch which proceeds inferiorly supplying branches to the central part of the upper two-thirds of the interventricular septum and penetrates the papillary muscle in the anatomical position of the moderator band and another which passes superiorly giving branches to the musculature of the upper part of the septum and ends in several small twigs in the region of the membranous septum close to the aortic root

In our experience the blood supply to the atrioventricular node and proximal three fourths to seven eighths of the atrioventricular bundle in the dog is from the posterior septal artery while the blood supply to the major parts of the right and left bundle branches and to the distal one eighth to one fourth of the atrioventricular bundle is from the anterior septal artery

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DISCUSSION

Our interpretation of the dynamics of these events is essentially as follows: the left ventricle contracts and delivers its stroke volume of blood into the aorta. This raises aortic pressure to its systolic peak. The auxiliary heart contracts at the beginning of diastole and delivers its stroke volume both cephalad and caudad into the aorta. This effectively raises the diastolic pressure in the aorta in two ways: 1) It pushes a quantity of blood into the thoracic and abdominal aortas. 2) It decreases the volume of the aorta available to contain this blood by the amount of collapsed aorta beneath the wrapped diaphragm. The result of these two effects is to markedly increase diastolic pressure and therefore mean pressure in the aorta. The increased aortic mean pressure results in an increased blood flow to all of the arterial branches including the coronary arteries. The coronary artery flow is particularly sensitive to increases in diastolic pressure as we have shown in previous work.[1] The homeostatic mechanisms of the animal respond to either this increased mean pressure or increased flow (either or both, this is not yet clear) by controlling the only part of the pumping mechanism under its control, i.e., the left ventricle. The mean pressure returns to normal. Normal mean pressures are now maintained by the left ventricle which works less vigorously with lower systolic pressures and by the auxiliary ventricle which maintains higher diastolic pressures. Because the left ventricular work is performed only during systole and is quantitatively related to the systolic pressures, decreased systolic pressures mean decreased ventricular work.

The left hemidiaphragm is adaptable for this purpose. It is a powerful muscle which can be mobilized at its periphery without disturbing the phrenic nerve or the base which contains its blood supply. Because its reaction time is short, it can function at the frequencies necessary. It is flat, can be folded easily and wrapped around the adjacent aorta. It is expendable as we and others have shown.[2] We have a series of dogs, to be reported at a later date, where the defect has been repaired with dacron cloth and the animals have survived with apparently no ill effects. In order to avoid the necessity of passing electrodes through the skin to the phrenic nerve, the feasibility of constructing an electronic device which would transmit a properly timed impulse through the intact skin is now being explored.

SUMMARY

1. This report describes exploration of methods to develop an auxiliary myocardium which would act in effect as a "booster heart."

2. The method consists of mobilizing the left leaf of the diaphragm peripherally in such a manner as to preserve its blood and nerve supplies. The muscle is wrapped around the distal portion of the thoracic aorta and stimulated during each diastole.

3. Measurements of arterial pressures and flows reveals that the auxiliary heart adds energy toward propelling the arterial blood peripherally.

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MULTICHAMBER PRESSURE RELATIONSHIPS IN EXPERIMENTAL HEART BLOCK*

NORMAN J SIDERIUS, PETER E BRUSSEN, GEORGE R DAICOFF,
JOSEPH E LANCASTER AND PETER V MOULDER

Acute heart block has proved to be one of the most troublesome complications resulting from intracardiac corrective surgery. Prevention awaits further surgical experience and anatomical localization studies in these abnormal hearts such as those of Lev.[1] Therapy at present depends upon anticipation with electrodes and pacemaker ready to produce electrical stimulation of the heart to maintain a reasonable ventricular rate.[2] This therapy is not always successful and more data on the dynamics of the effects of the acute atrioventricular heart block are necessary. Since this acute heart block occurs in the operative or early postoperative period when the precise status of the blood volume of these patients is difficult to determine, this factor is a major facet and the only variable to be considered in this preliminary report.

METHOD

Mongrel dogs weighing 15 to 25 kg, receiving no preparatory medications, were anesthetized with intravenous thiobarbiturate compounds. Intermittent manual positive pressure breathing was done via cuffed endotracheal tube using a closed system (McKesson) with a fresh CO_2 absorber (soda lime). Through a standard right thoracotomy stiff polyethylene catheters (I D 1 57 mm) of equal length (100 cm) were inserted into both atria, the pulmonary artery and the central aorta. In some experiments the atrial catheters were so placed that slight advancement allowed them to enter the ventricular chambers for recording of these pressure pulse curves. Blood pressures were recorded via Statham P23D strain gauges simultaneously on four channels with electrocardiogram (Lead II) on a Grass direct writing Polygraph. Although continuous observations were made, the data used in this study were made in set periods in steady state with minimal or no respiratory motion. Acceptable data with these restrictions could be collected from only six experiments, but the remainder of the partial or continuous studies on more than 30 dogs followed the patterns to be described herein. Mean pressures were obtained by planimetric measurements.

Heart block was produced in 4 dogs by surgical division of the atrioventricular node site during temporary venous occlusion through a right atriotomy and in 2 by constriction with a previously placed ligature in the same area following restitution of the circulation.

A series of recordings were made following venous inflow occlusion and atriotomy alone (normothermia) on 5 dogs to obtain a control for this strenuous procedure.

RESULTS

Effect of Venous Inflow Occlusion and Atriotomy During a 3 minute period of venous inflow occlusion the intracardiac pressures fell to near zero. Two to

* From the Department of Surgery, The University of Chicago. Supported by a grant in aid from the Chicago Heart Association.
With the technical assistance of Mr William Rank.

3 minutes after reestablishment of the circulation there was a mild trans hypertension in all chambers At a steady state time comparable to the "im diate postblock" period to be described in the next section, the only ch was a diminution in the aortic pressures There was no significant chang the heart rate These findings are graphically illustrated in Figure 1

Immediate Effect of Acute Complete Heart Block. All cardiac chamber the animals with acute heart block were markedly dilated The ventric contractions appeared strong and the aorta and pulmonary artery eviden this thrust with great distention following systole The peripheral pulse 'pistol shot" in character

A reasonable state was reached in these experiments 2 to 3 minutes a restoration of the circulation which allowed assessment of the immediate p block period (Fig 2) The ventricular rate was about one third of preblock value The aortic diastolic and mean pressures were markedly duced, but the systolic pressure fell minimally The pulmonary arte systolic pressure rose, while the diastolic and mean pressures were unchang [illegible] were increased

[illegible] The ventricular rate mained about 50/minute Over the variable period of 5 to 60 minutes th was a return toward normal of the pressure values in the aorta and pulmon arteries although this final relatively stable set of pressures did not reach preblock levels as has been graphically presented in the third column Figure 2 The elevated atrial pressures remained about the same

Effect of Hypervolemia in Acute Heart Block. The relatively rapid additi of 500 cc whole blood, about one third of the normal blood volume, to t vascular system of 3 dogs in a stable state postblock produced a striking f ther distention of the atrial chambers and an elevation of all pressures (exce aortic systolic) and especially the mean pressures (Fig 2) There was no crease in the ventricular rate

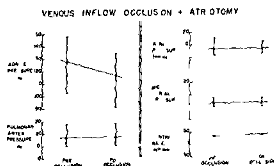


Fig 1 Simultaneous blood pressure values obtained before and after recovery from 3 minutes of venous inflow occlusion and atriotomy alone (average of 5 dogs)

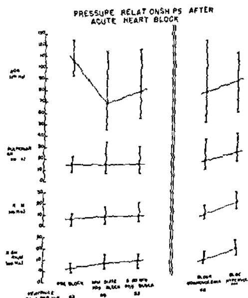


Fig 2 Simultaneous blood pressure valu obtained before and after acute comple heart block (average of 6 dogs) The figur on the right demonstrate the effect of hype volemia on blood pressures in heart bloc (average of 3 dogs)

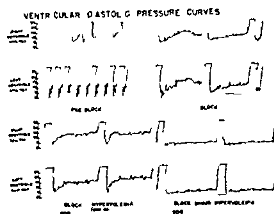


Fig 3 An example of simultaneous right and left ventricular diastolic blood pressure curves before and after acute complete heart block The effect of the addition and removal of 500 cc blood on these diastolic levels during block is shown in the lower panels

The Effect of Heart Block on Ventricular Diastolic Pressures In heart block the ventricular diastolic filling time was increased (Fig 3) The contributions of the repeated atrial contractions during the ventricular diastolic phase were observed Increasing the blood volume increased both the left and right ventricular diastolic pressures Comparison of atrial and ventricular diastolic curves taken successively rather than simultaneously in a number of instances showed a close correlation suggesting that the elevation in atrial pressure was primarily a reflection of the ventricular pressure

DISCUSSION

These preliminary observations corroborate and extend the findings of Starzl and Gaertner[3 4 5] and Mowlem and Campbell[6 7] although the initial postblock elevations of mean left atrial pressure were considerably less than those in the studies of Mowlem and Campbell These studies indicate that the elevated diastolic pressures in the 2 atria are a reflection of ventricular diastolic elevations rather than evidence of relative atrioventricular valvular stenosis One of the most significant observations in this study is the congestion and dilatation of all chambers following acute heart block Furthermore, any blood volume excess exaggerates the chamber dilatation and increases the pressures in them without any favorable effect on ventricular rate or mean aortic pressure

With the decreased cardiac output shown by Starzl a hypovolemia would be deleterious for peripheral and coronary circulation thus emphasizing the need for normovolemia after intracardiac surgery

CONCLUSION

1 Acute complete atrioventricular heart block with one third of normal ventricular rate (=50/min) produced an immediate elevation in the pressures of the atria and pulmonary artery and a fall in the aortic pressure The later (5 to 60 min) stabilized effect was less in the aorta and pulmonary artery but the same in the right and left atria

2 Additional blood volume did not influence the ventricular rate, exaggerated the cardiac distention and markedly increased all pressure parameters in the four chambers and pulmonary artery

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THE FATE OF POTASSIUM AS A CARDIOPLEGIC AGENT IN NORMOTHERMIC ANIMALS *

ABRAHAM KAPLAN, MORTON LEVINE AND BERNARD FISHER

The desirability of a motionless, dry surgical field during open cardio
cannot be denied At the present time, in spite of the attractive featu

bility of cardioplegia vary from those of total skepticism and discourage
of its use to those who use it constantly with remarkable success The
discrepancy exists as to which cardioplegic agent is the best

Bjork [1] was unable to induce reversible cardiac arrest by either local
ing or by the injection of acetylcholine On the other hand Niazi and L
demonstrated the ability of the rat heart to survive prolonged perio
cardiac arrest when body temperatures were in the vicinity of 5°C Ventri
fibrillation in dogs was induced by Senning [3] at the same time maintain
circulation by means of a pump It was felt by him that prolonged peri
... were well tolerated under such circumstances, and he emph

occlusion and he favors acetylcholine to accomplish this Lillei, who,
extensive clinical experience, is an enthusiastic advocate of the use of
sium induced cardioplegia

Knowledge that cardiac action can be stopped and reestablished by c
cals is not newly acquired Basic observations were made in 1883 by Ri
and the real foundation of present techniques of cardioplegia during
heart surgery were developed by Hooker and Wiggers,[7, 8] who demons
the effects of electrolyte solutions as cardioplegic agents during fibrill
More recently, Melrose [9] has given new impetus to the use of potassium
cardioplegic agent by describing a simple technique for clinical applic
... rapid developments in the field of cardia

agents and our physiologic knowledge ...
the metabolism of potassium injected as a cardioplegic agent ...

* From the Department of Surgery Laboratory of Experimental Surgery Univer
Pittsburgh Medical School Pittsburgh Pa Supported in part by the Western Penns
Heart Association

undertaken under a variety of circumstances, i.e. normothermia, hypothermia, coronary perfusion and with the pump oxygenator. Further, studies of changes in cell membrane potential produced by the use of this agent are in progress. It is the purpose of this paper to present information obtained from normothermic dogs concerning the fate of potassium (K^{42}) when used as a cardioplegic agent.

METHOD

Mongrel dogs of both sexes weighing 10 to 15 kg. were used. They were anesthetized with intravenous nembutal, 35 mg./kg., and connected to an automatic respirator. Bilateral thoracotomy was performed, the azygos vein ligated, and both cavae were prepared for venous inflow occlusion. Following the latter, and clamping of the aorta, cardiac arrest was accomplished by the injection of a solution of 25% potassium citrate (2 ml.), normal saline (18 ml.), and heparin (0.5 ml.), containing K^{42}.† Because of its short half-life (12½ hours), K^{42} was added to the solution in varying volumes depending upon its age; i.e. 0.02 ml. the day of arrival, 0.06 ml. the second day and 0.1 ml. the third day. The activity of the solution to be injected was determined by placing the loaded syringe under the scintillation tube just prior to injection.

During cardioplegia and the entire process of resuscitation radioactivity in the heart was measured by a sodium iodide, thallium detector for gamma rays (Nuclear-Chicago) which fed into a count-rate meter and this in turn into a linear and logarithmic recorder. Before inducing cardioplegia, the detector was placed over the heart and background was recorded. A collimator was used on the detector to eliminate activity of tissue surrounding the heart. The distance between the heart and detector was calculated by diagramatically projecting a cone of radioactive area from the detector and then determining the level of the cone at which the dimensions of the heart of each animal best fit, (Fig. 1). This distance, measured in centimeters was used during the experiment. It was felt that the geometry of the arrested heart and the beating one changed little. This factor was therefore considered negligible.

At the onset of cardioplegia, blood and the injected potassium solution which accumulated in the right atrium was removed by needle aspiration. The volume withdrawn and radioactivity of an aliquot was determined. Also, at this time a piece of myocardium ("M_1") was removed from the right ventricular wall without opening into the heart. Although this experiment was designed to duplicate the clinical techniques of open heart surgery with elective cardiac arrest as closely as possible, actual cardiotomy with its attendant spilling of K^{42} into the chest cavity and loss of radioactivity was not performed.

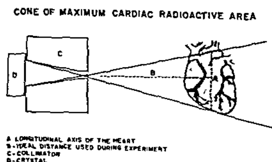


Fig. 1.

† From Oak Ridge National Laboratory (10 millicuries in approximately 1 ml. of solution.)

With reestablishment of venous inflow and the onset of cardiac message, elimination of radioactive potassium from the myocardium was recorded, and simultaneously blood samples were taken from a femoral artery for measurement of K^{42} in the peripheral blood (500 lambda of plasma) When a stable plateau of counts from the heart, which had or had not resumed beating was obtained, a second sample of myocardium, (M_2) was removed and the experiment was terminated All animals were then sacrificed and the hearts were removed and weighed The radioactivity and that of the lungs was determined separately *in vitro* to eliminate the possibility that activity from the latter had been recorded *in vivo* by the detector over the heart Sections of lung liver and kidney were likewise taken for determination of radioactivity Blood samples were taken for determination of pH and serum potassium before and after surgery and the ECG was continuously monitored

RESULTS

An average of 10 ml of potassium solution with a mean activity of 4,905 619 counts per minute was required to produce cardioplegia in the 12 animals used in this study A typical tracing of radioactivity detected over the heart *"in situ"* is shown in Figure 2 Following a sharp ascending limb which depicts the injection of potassium into the aorta, radioactivity was seen to remain fairly constant during the period of vascular occlusion A decrease in counts recorded occurred following aspiration of the right atrial blood The relationship between the amount of activity detected in the atrial sample to the amount of radioactive substance injected was quite variable An average of 9%, (0 4%–30%) of the K^{42} injected was found in the blood, (12 ml) accumulated in the right atrium

With reestablishment of the venous inflow and removal of the aortic clamp (after 6 minutes occlusion) activity detected over the heart was reduced to 62% of that maximally obtained during occlusion Following a few seconds of cardiac massage, a further decrease occurred, but 31% of the original activity was still present in the heart Following this a plateau was reached which remained stable and practically unmodified for the rest of the experiment Signs of electrical activity were recorded when 29% of the K^{42} injected was still in the heart and an average of 30% of the activity was present at the termination of the experiment

The first sample taken from the myocardium (M_1) was considered representative of the amount of potassium picked up by the heart during venous inflow occlusion This was found to average 19 380 counts/gram of heart muscle By comparing this value with the radioactivity of a second sample of muscle (M_2) at the termination of the experiment (average of 6 895 counts/gram of heart muscle) it was possible to estimate the amount of

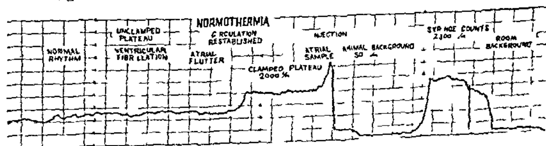


Fig 2

potassium eliminated from the myocardium during the period of resuscitation. There was a remarkable agreement between the amount of potassium retained by the muscle (36%) and that determined by scanning over the heart. No relationship between the amount of residual activity and resumption of normal cardiac rhythm was found. In 3 of the 12 animals, normal rhythm was never resumed and yet the percentage of potassium eliminated from the myocardium was 88, 91, and 95%. Similarly, animals resumed normal heart beat with as low as 32% elimination of potassium.

Evidence that circulation was effectively reestablished before termination of the experiment was obtained from the fact that K^{42} was equally distributed throughout the body, there being a close correlation between the number of counts per gram of liver, kidney and lung. For example, in Dog 9 these were 1,915 counts/gram of lung, 1,366 counts/gram of kidney, and 1,366 counts/gram of liver.

Following release of occlusion clamps, an increase in K^{42} in the samples of peripheral blood was obtained abruptly—the maximum count being obtained within 2 to 3 minutes There was then a gradual decline in the number of counts obtained per sample. At termination of the experiment, final

Table 1 Normothermic Dogs—Averages of 12 Experiments

K Citrate Injected (Ml)	10	Time of appearance of electric activity	3 min
Radioactive K^{42} Injected (counts/min)	4,905 649	Time of appearance of sinus rhythm	8 min
Ml from Atrium	12	Radioactivity of Lung (counts/gram)	2,536
Radioactivity (counts/min)	236,196		
Radioactivity of M_1 (counts/gram)	19,088	Radioactivity of Kidney (counts/gram)	2,262
Radioactivity of M_2 (counts/gram)	6,895	Radioactivity of Liver (counts/gram)	2,050
Residual activity in Myocardium (%)	36	Time of occlusion	6 min
Disappearance of K^{42} from peripheral blood (%)	33	Mortality rate	25%

Table 2. Normothermic Dogs—Radioactivity Over the Heart—Relationship Between Number of Counts and Different Stages During Standstill and Resuscitation

VENOUS INFLOW OCCLUSION	VENOUS INFLOW REESTABLISHMENT	DURING MASSAGE	SIGNS OF ELECTRICAL ACTIVITY	END OF EXPERIMENT
100%	62%	31%	29%	30%

peripheral blood samples still demonstrated an average of 66% of the concentration of potassium obtained in the first peripheral blood sample Again it may be emphasized that no correlation between the concentration of potassium in peripheral blood and resumption of normal rhythm could be made

No change in serum potassium or pH was found before or after occlusion These values were obtained in order to eliminate two of the more important factors which could influence failure of the heart to return to normal rhythm

DISCUSSION

No uniform pattern has occurred in these experiments It is impossible to relate the amount of potassium injected to the reversibility of the cardioplegia In general, it may be said that results indicated that the more potassium used, the greater the possibility that recovery would not occur (Experiments 5 and 7)

It would seem from our observations that perhaps the injected potassium becomes distributed in the heart into two 'compartments , one from which it is eliminated rapidly and from the other more slowly It is suggested that the one which is more readily removed is the potassium accumulated in the vascular and to some degree the interstitual space That which is more slowly eliminated in all probability is potassium which has entered the myocardial cell The latter is based upon the findings that the concentration of radioactivity at the end of the experiment in a piece of myocardium (M_2) is still 30% of the maximum activity found at the onset of cardioplegia

The reversibility of the phenomenon of cardioplegia is not solely related to the concentration of potassium in the myocardium, for the same stable plateau of radioactivity may be observed with hearts in fibrillation, demonstrating just slight electrical activity, or in those completely recovered Once resuscitative measures are instituted, it might properly be speculated that a derangement of the normal architecture of electrical potentials is set off by the cardioplegia which requires more than the measures we now institute to reestablish the dynamic equilibrium of the myocardial cell It is very likely that the safety of a cardioplegic agent in the future will depend entirely upon there being a specific anticardioplegic agent which will be equally effective in re establishing a normal physiologic state

SUMMARY AND CONCLUSION

This experimental work has attempted to establish with the aid of K^{42}, (a) the qualitative relationship between the amount of potassium injected and the reversibility of the phenomenon of cardioplegia, (b) the distribution and fate of the injected potassium, (c) its rate of elimination from myocardium and peripheral blood and (d) the correlation if any between the concentration of potassium in the myocardium and normal or abnormal rhythms

It has been concluded that

1 No uniform relationship exists between the amount of potassium injected and the reversibility of cardioplegia although high concentrations of potassium will compromise the possibility of recovery

2 The elimination of potassium is probably not the only factor in resumption of the heart beat

3 According to its behavior during resuscitation, injected potassium is probably distributed into two "compartments", (a) a fast elimination

compartment from which it is easily removed during resuscitation and (b) one from which there is slow elimination but the removal from which did not necessarily mean the reestablishment of normal rhythm

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THE IMPLICATION OF EXCESS OXYGEN AS A FACTOR IN CARDIAC ARREST *

Sam E Stephenson Jr

Observations from experiments designed to evaluate an electronic respirator control † which has previously been described [1, 2] (Fig 1) were at first discouraging since anesthetized animals using room air and their own biochemical demands to control the rate and depth of respirations fell into a level of arterial oxygen saturation which we would clinically accept as hypoxia The experimental animals however maintained an essentially normal arterial pH

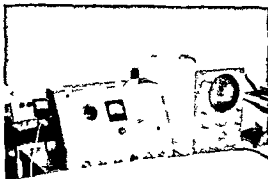


Fig 1 The Electronic Respirator control On the left is a transistor unit adjacent to the conventional tube model The oscillo scope is useful in selecting the satisfactory operating frequency The control can be at tached to any respirator with minor modi fication

† Developed in conjunction with the Department of Anatomy F H Montgomery Dr S L Clark and Dr R Batson

* From the S R Light Laboratory for Surgical Research the Departments of Surgery and Pediatrics and the Vanderbilt Poliomyelitis Respiratory and Rehabilitation Center Vanderbilt University School of Medicine Nashville Aided by a grant from The National Foundation

Table 1

	CONTROL	ANOXIA	RESPIRATOR 3	1 HR	3 HR	5 HR
Group A ph	7.25	−.07	—	+0.016	+0.007	—
CO_2 Ten	57 mm	+9 mm	—	−9 mm	+3.03 mm	—
O_2 Sat	85.56%	−11.51%	—	+5.53%	+8.51%	
Group B ph	7.22	−.18	+.005	+.056	+.004	−.015
CO_2 Ten	54.5 mm	+5	−.66 mm	−5.5 mm	−3.8 mm	+7.5 mm
O_2 Sat	74.64%	−14%	+4.5%	+3.38%	+1.22%	+7.4%

Average results of a group of experimental animals dependent on the respirator for a prolonged period of time. Group A is composed of animals with chronic respiratory insufficiency and Group B animals were subjected to bilateral thoracotomy and phrenicotomy.

and pCO_2 on a straight line relation for periods up to 3 hours while under going bilateral thoracotomy and simultaneous bilateral phrenicotomy (Table 1). As data were collected on a large group of experimental animals these low arterial oxygen saturations under anesthesia were a constant finding. Following this, an experimental study was undertaken to determine the role played by (1) the anesthetic agent, (2) physiological oxygen saturation in inspired air, and (3) increased O_2 tension in the inspired air. The principle data in this report developed as an ancillary observation from the above studies.

The prolonged administration of oxygen enriched air to animals under the conditions of this experiment brought about marked changes in arterial blood studies and, in fact, instances of cardiac arrest in what appeared to be a healthy myocardium.

METHOD

Unselected mongrel dogs of 6 to 16 kg were used in the study. Under local procaine anesthesia the right femoral artery was cannulated for arterial blood sampling using anerobic techniques. After control samples were obtained the animal was anesthetized with 30 mg/kg of pentobarbital sodium in most cases. A cuffed oropharyngeal endotracheal tube was inserted. A constant recording of the electrocardiogram was obtained with conventional leg leads and the electronic respirator control was attached by using indifferent and ground electrodes placed at random in the skin. The active #24 gauge hypodermic needle electrode was inserted into one ala nasi. The respirator control activated a Monoghan conventional blower at 20 mm. of Hg positive pressure and 3 mm. of negative pressure. This was connected to a nonrebreathing valve with a side arm and thence to the endotracheal tube.

Bilateral third interspace thoracotomies and phrenicotomies were performed. The animals were then divided into three groups for study. pH determinations were made on a Beckman pH meter utilizing an anerobic cell. pCO_2 and O_2 saturations were performed with a manometric Van Slyke apparatus. Oxygen saturation of the inspired air was measured with a Beckman oxygen meter.

RESULTS

Group I consisted of 6 animals which were carried on room air for 1 hour given oxygen enriched air for an hour and allowed to recover using room air. The change in arterial studies using 25% and 30% oxygen was acceptable. When the inspired air was enriched to 35% oxygen, however, the average change in pH on these 3 animals was a drop from 7.33 to 7.07 which was accompanied by average pCO_2 elevation from 41 mm Hg to 75.5 mm Hg and oxygen saturation increased from 83.5% to 100.5% (Fig 2). Accompanying these changes there was electrical instability of the electrocardiogram. The animals were then shifted back to room air and given 1 hour to recover and at the end of this time there was no significant change in any of the studies from the baseline.

Group II consisted of 6 animals which were given oxygen enriched air for 1 to 3 hours following baseline studies and the survivors were allowed to recover using room air. The Group II results concerning 25% and 30% oxygen concentrations were similar although minor changes were present. All animals recovered and returned to normal on room air. Of the 4 animals whose inspired air contained 35% or more oxygen 3 expired from cardiac arrest developing in a well oxygenated heart after 2½ hours, 2 hours and 2 hours respectively. The average pH change was from 7.36 to 7.17 at 1 hour and 7.06 at the end of 2 hours. The pCO_2 elevated from controls of 42.9 mm Hg to 54 at 1 hour and 74+ at the end of 2 hours. Oxygen saturation increased from 86.6% to 95.2% and finally to 102% on the average.

The 3 animals constituting Group III or those animals subjected to complete transection of the cord and allowed to react from anesthesia before they were given oxygen enriched air showed only minor deviations. There was on the average no change in pH. pCO_2 was elevated 1.4 mm Hg and oxygen saturation increased 1.3%.

The data from Group III seem to implicate the anesthetic agent as the prime factor allowing the previously mentioned changes to occur. Barbiturate anesthesia was chosen for its marked depression on the respiratory pathways and one would assume that these changes would be more marked than under ether or cyclopropane.

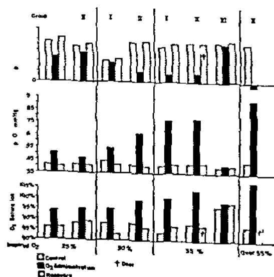


Fig 2 Average changes occurring in a group of 15 experimental animals. Group I, II and III coincide with the text description.

Figure 3 shows a typical tracing of one of the animals developing *cardiac* arrest and one can see the marked instability of the EKG prior to arrest

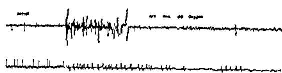


Fig 3 Tracing from an experimental animal receiving 40% oxygen in the inspired air The upper tracing is the electroencephalogram and the lower the electrocardiogram

DISCUSSION

The instability of the heart both physiologically and electrically is difficult to measure but observations on cardiac rhythm between an over oxygenated heart and a hypoxic (not anoxic) heart is quite striking In animals with arterial oxygen saturations of 86 to 92% accompanied by *normal* pCO and pH the rhythm is slow regular and each beat forceful If an animal under these conditions is subjected *to a pneumonectomy there is no disturbance in cardiac* activity in fact it is rare to observe a single ventricular premature contraction On the other hand if the same *procedure is carried out on an animal* breathing 30 to 40% oxygen or even subjected to simple hyperventilation the electrocardiogram is quite unstable and any *manipulation around the hilus* of the lung causes runs of premature contraction and ventricular tachycardia

The data of the Group II animals showing the 3 dogs in which cardiac arrest occurred in pink normal appearing hearts are quite distressing when *one considers the degree of hyperventilation excess oxygenation* and wanton changing of oxygen concentration that occurs during anesthesia for surgical *procedures The animals subjected to complete division of the spinal* cord between C_1 and C_2 serve as a control for this and seem to clearly implicate *the anesthetic agents as the solitary change which brings about this* ability to overoxygenate As one can see from the results the addition of excess amounts of oxygen *in comparison to atmospheric air* makes *little or* no change in arterial blood determination *in the awake* animal These changes are *prevented by a slowing in the respiratory* rate and a *reduction in the* depth of respiration

This experimental preparation is unique in that we *know of no other* available instrument which will allow an animal totally dependent on a respirator to control *each breath by his own* biochemical needs This fact may be responsible *for these changes not previously* being noted A possible explanation is that the barbiturate anesthesia employed *is known to depress the respiratory*

center

stimulu

might then depress these ancillary centers and allow the shift in pH and pCO

The question *in* our minds has been the approach *to the* anesthetic state Should not a patient under general anesthesia respond more like an individual engaging in *normal sleep than like an alert* awake one? Very little data are available in the literature concerning studies of arterial oxygenation pCO_2 and *pH during sleep Droust*[3] *and co-workers report* oxygen saturations of 88 to 92% during normal sleep and Mills[4] reports a 2 to 4 mm elevation of alveolar CO tension *during sleep* With these previously reported studies and our own observations we feel that a reconsideration of what we wish to accomplish *under anesthesia in regard to oxygenation* is in order and that the associated fall of pH and elevation of CO_2 tension which can occur with

overoxygenation may be the precipitating factor in some previously unexplained cases of cardiac arrest

Many facets of this problem still need to be explained The changes occurring with volatile anesthetics follow the same trend but are not as marked as those changes occurring with barbiturate anesthesia The combination of light anesthesia and muscular relaxants is difficult for us to compare since we depend on a contracting muscle to furnish the stimulus to trigger our respirator A similar implication of overoxygenation or in fact oxygen intoxication has been reported by Kolff[5] in conjunction with extracorporeal circulation Studies along these lines are continuing

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HIGH ENERGY PHOSPHATE METABOLISM IN THE MYOCARDIUM DURING VARIOUS TECHNIQUES OF CARDIAC ARREST AS DETERMINED BY CARDIAC BIOPSY *

VINCENT L GOTT MARILYN BARTLETT JOHN A JOHNSON

Induced cardiac arrest has become a standard procedure at most centers where open heart surgery is being done with a pump oxygenator Potassium citrate as advocated by Melrose[1] and acetylcholine as proposed by Lam[2] are the most widely used cardioplegic drugs at the present time Despite the successful clinical utilization of these drugs virtually no information is available regarding the fate of high energy substances of the myocardium during the total ischemia that accompanies cardiac arrest Determination of these high energy substances during arrest should aid in more properly evaluating the period of anoxia that the myocardium can tolerate safely

The most important energy reservoirs of the myocardium are the high energy phosphates adenosine triphosphate (ATP) adenosine diphosphate (ADP) and phosphocreatine (PC) ATP is the essential fuel for all types of tissue activity ranging from muscular contraction down to maintenance of cellular viability As ATP is utilized ADP is formed which in turn may be recharged back to ATP by at least three important processes The first process is the donation of a high energy phosphate radical from the substance phosphocreatine Phosphocreatine is a storehouse of high energy phosphate which is readily available when the ATP level falls A second source of high energy

* From the University of Minnesota Medical School Supported in part by a Minnesota Heart Association Grant

phosphate results from the anaerobic breakdown of glycogen to lactic acid and the third source results from the further breakdown of lactic acid to H_2O and CO_2 via the Kreb cycle In normal physiological conditions the last mechanism is probably the main source of high energy phosphate Because this mechanism requires the presence of oxygen, it is severely limited as a generator of high energy phosphate during the complete ischemia of cardiac arrest The myocardium therefore must depend mainly on the first two processes to maintain an adequate level of ATP

In this study the myocardial muscle was analyzed for ATP, ADP, phosphocreatine and glycogen during one hour of cardiac arrest, induced either by potassium citrate or acetylcholine

METHOD

Adult mongrel dogs were anesthetized with pentothal and the hearts were arrested with 2½% potassium citrate in blood or with acetylcholine (10 mg /kg of body weight) [2] In order to maintain the hearts at a constant temperature they were removed from the dog at the time of arrest and placed in a plastic chamber maintained at 37°C Small myocardial biopsies were taken with a special instrument that allowed freezing of the muscle with liquid nitrogen as it was being removed from the heart All biopsies were kept in liquid nitrogen and all weighing and initial handling of the tissue was performed in a cold room at —20°C

The inorganic phosphate (IP) was determined by a modified Lowry and Lopez technique [3] A Fiske and SubbaRow [4] phosphate determination was made on an aliquot of the extract and the increase over the Lowry and Lopez determination was taken as the phosphate of phosphocreatine A duplicate aliquot of the extract was then made 1 N by the addition of sulfuric acid and boiled for 12 minutes The increase in Fiske and SubbaRow phosphate was taken as the labile phosphate linked with adenosine or the high energy phosphate of ATP and ADP combined ADP was determined specifically by the technique of Reynard and Boyer [5] Since 1 mM of ADP liberates 1 mM of high energy phosphate on acid hydrolysis, the concentration of ADP in mM was subtracted from the total acid labile phosphate to determine the amount of ATP Glycogen was determined by the techniques of Stadie *et al* [6] and Benedict [7]

RESULTS

The data obtained from hearts arrested by potassium citrate is shown in Figure 1 These are the mean values of 8 arrested hearts The mean values obtained on 5 hearts arrested by acetylcholine are shown in Figure 2

In comparing phosphocreatine levels during the two types of arrest, it may be noted that in acetylcholine arrest the phosphocreatine dropped to a low level twice as fast as hearts arrested with potassium citrate It is important to note that hearts injected with acetylcholine continued to beat very slowly as it is impossible to completely arrest them Bradycardia continued in these hearts for about ten minutes at which time the hearts appeared to arrest and the phosphocreatine had dropped to a low level The ADP level in both types of arrest rose only slightly and remained fairly constant throughout the period of arrest This is further evidence that several mechanisms are working to return the ADP that is formed back to ATP The ATP level does fall progressively throughout both types of arrest but does not drop to more than 50%

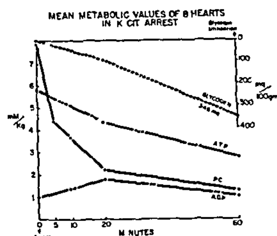


Fig 1 Mean metabolic values of 8 hearts in potassium citrate arrest

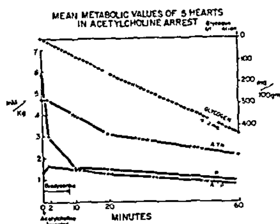


Fig 2 Mean metabolic values of 5 hearts in acetylcholine arrest

of the initial value It may be noted in Figure 1 and 2 that the glycogen content of the myocardium fell more rapidly in the hearts arrested with acetylcholine Although there is wide variation in the glycogen content in different areas of the myocardium, the difference in the glycogen utilization in the two types of arrest is statistically significant

DISCUSSION

When the workload is completely removed from the beating heart by cardiopulmonary bypass and biventricular cardiotomies the energy requirements drop to 15% of normal If these same hearts are then arrested completely with potassium citrate, the energy requirements are reduced an additional 50% or to 7% of the normal working heart This data explains in part why the totally ischemic human heart will sometimes tolerate periods of up to one hour of induced cardiac arrest It is known, however, that the safe period of induced arrest in the human heart is usually well under one hour, and in the dog heart survival is rare if the arrest lasts longer than 20 to 30 minutes There are many factors that could contribute to the success or failure of induced cardiac arrest Changes in the cation and anion level, in the pH, and in the contractile proteins are all conceivable factors Probably of importance in the success or failure of arrest is the degree of breakdown of the high energy compounds

Possibly the depletion of phosphocreatine after 20 minutes of potassium citrate arrest explains in part the low survival rate for canine arrests which exceed this time period After the phosphocreatine reaches low levels the myocardium is dependent to a large extent on glycolysis as a source of high energy phosphate This leads to an excess of lactic acid with the accompanying deleterious pH changes and possibly an irreversible condition Because acetylcholine arrest seems to allow an even higher metabolic rate with a more rapid depletion of phosphocreatine and glycogen, the safe preiod may be even briefer than in potassium citrate arrest

CONCLUSIONS

1 In hearts arrested with potassium citrate the readily available storehouse of high energy phosphate contained in phosphocreatine drops to low levels

in 20 minutes In hearts arrested with acetylcholine the phosphocreatine drops to low levels in 10 minutes

2 The glycogen breakdown was more rapid in acetylcholine arrest than in potassium citrate arrest

3 The ADP in both types of arrest remained low and constant, indicating that ADP was being recharged to ATP probably by glycolysis and phosphocreatine breakdown

4 The ATP level in both types of arrest dropped only 50% during one hour of arrest

5 It would appear that the safe period of potassium citrate arrest in dogs is under 20 minutes and that potassium citrate arrest is more satisfactory than acetylcholine arrest from the viewpoint of maintenance of high energy phosphate levels

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METABOLISM OF THE ISOLATED NORMOTHERMIC AND REWARMED HEART*

SAE S LEE AND WATTS R WEBB

It has been demonstrated previously that the isolated mammalian heart can survive for prolonged periods at low temperatures and return to adequate clinical function [1] The present study was designed to determine the metabolic changes of the heart on restoration of function after periods of ischemia and refrigeration

METHOD

Donor hearts were obtained from small dogs which had been heparinized with 10 mg of heparin per kg of body weight The hearts were either immediately transplanted to the neck of large recipient dogs or immersed in chilled Tyrode s solution with 10% serum at 4°C for a period ranging from 6 to 8 hours The distal carotid artery of the host was anastomosed to the donor s brachiocephalic artery and the proximal end of the recipient s jugular vein [illegible] of the donor [illegible] some experiments to test the effect of the addition of a work load After restoration of normal cardiac function samples of blood were taken from the carotid artery and timed samples collected from the pulmonary artery of the graft This

* From the Department of Surgery University of Mississippi Medical Center Jackson
Supported by National Institutes of Health Grant Number H 2806(C)

latter afforded a measure of the coronary flow which had emptied into the right ventricle. Samples were obtained over several hours *with and without* the addition of the heavy work load from the proximal carotid artery, which approximated normal cardiac output.[1] The blood glucose, lactic acid, pyruvate, O_2, and CO_2 values were determined. After completion of the experiment, the left ventricles were weighed after trimming away all other tissues.[2]

In the first series the hearts were transplanted immediately after excision. In the second series the excised hearts were refrigerated for a period of 6 to 8 hours, and subsequently transplanted. In the third series the hearts were refrigerated and transplanted as in the second series but 500 mg. of thiamin and 500 mg. of nicotinamide were given intravenously to the recipient dog about 30 minutes prior to the transplantation.

RESULTS

Series I. The 7 hearts immediately transplanted returned to adequate function in all instances. Occasionally it was necessary to use an electric defibrillator, but more often the heart spontaneously resumed a normal sinus rhythm. The coronary blood flow averaged 128 cc./100 gm. left ventricle, with a rise to 313 cc. with the addition of a work load. The O_2 consumption averaged 6.4 cc./100 gm./min. and CO_2 averaged 7.1 cc. for an R.Q. of 1.1 with a range from 0.7 to 1.2. The transplanted hearts immediately metabolized glucose, lactate, and pyruvate, averaging approximately 11 mg. of glucose, 10 mg. of lactate, but minimal amounts of pyruvate (0.7 to 2.6 mg.). Four of the 7 hearts showed a negative balance of pyruvate at some time, usually early with improving utilization later. The metabolism of all was increased by adding a

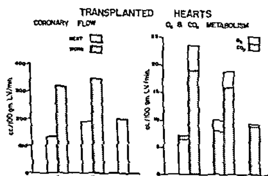


Fig. 1. Chart of averages of coronary flows and O_2 and CO_2 utilization by transplanted heart. I immediately transplanted hearts. II refrigerated hearts. III refrigerated hearts with thiamin and nicotinamide.

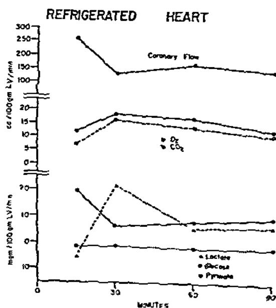


Fig. 2. Chart of transplanted heart (Group II) showing changes with time. Note the high coronary flow with stabilization after 30 minutes. CO_2 and O_2 consumption likewise show high values early. Lactate showed a negative balance early with later utilization. Glucose utilization diminished, perhaps as more lactate was metabolized.

work load or by increasing the pulse rate but this could not be quantitated

Series II. In these experiments to warm the heart and restore normal function it was necessary to massage the heart for long periods and use adrenalin or calcium injections to increase tone and rhythmicity Nearly all required electric defibrillation Coronary blood flow in this group averaged approximately 188 cc /100 gm of weight An average of 10 cc of O_2 were utilized and 8 cc of CO_2 produced with an R Q of 0 8 Glucose metabolism usually approximated that of Group 1, except for occasional instances of higher glucose levels in the coronary venous blood Lactate utilization was diminished initially with higher levels in the coronary venous blood than in the arterial blood Later specimens usually showed increased lactate utilization There was little or no metabolism of pyruvic acid

Series III. These hearts which were transplanted to the dogs having large injections of thiamin and nicotinamide clinically did much better than those not receiving vitamin therapy They restarted easier, defibrillated easier, and appeared much stronger The metabolism was very similar to that of the hearts in Series II after the latter had returned to excellent condition Oxygen and CO_2 utilization were 9 2 and 8 8 cc /100 gm left ventricle respectively Coronary flow averaged 199 cc /min Utilization of glucose and lactic acid was approximately that of the control, while 3 of the 6 hearts did utilize pyruvate in a definite improvement over Group II

DISCUSSION

In the first series of immediately transplanted hearts which had had 30 to 50 minutes of anoxia, the myocardium metabolized glucose, lactate and pyruvic acid with definite improvement being shown with time In the re

Occasionally the glucose levels in coronary venous blood exceeded those of the arterial blood Bing[3] observed that in arrested hearts the glucose concentration in coronary venous blood might exceed that in the arterial perfusate by as much as 70 mg % Lactic acid also was in negative balance immediately after restoration of the heart, probably due in part to lactate accumulation during the period of anoxia

After periods of refrigeration ranging from 6 to 8 hours the hearts showed a diminution to absence of pyruvate metabolism In addition, glucose and lactate metabolism, while often normal might be reduced and frequently the glucose and lactate concentrations in the venous blood were elevated above those in the arterial blood Govier[4] and Grieg[5] demonstrated destruction of various enzymes during shock and anoxia With the intravenous injection of thiamin and nicotinamide, the drugs pass to the recipient dog's liver to be converted rapidly into co carboxylase and co enzyme These appear to restore the aerobic metabolism of pyruvic acid

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by introducing water into the closed system and circulation is maintained at a rate of 2 L/min by the sigmamotor pump

During the cooling procedure the temperature of the fluid circulating through the refrigerating blankets is —2°C and that of the water in the balloon 8°C The rewarming temperatures are 46°C in the blankets and 41°C in the balloon The temperature of the gastric balloon can be rapidly altered when necessary by placing the external coils of plastic tubing in water of the desired temperature

Eleven mongrel dogs were subjected to experiments designed to determine the degree of safety with which a gastric balloon might be used in hypothermia Comparisons were made between the rates of rectal temperature change in dogs cooled and rewarmed solely with a refrigerated blanket and with blanket and gastric balloon combined The results were as follows 1) cooling with blanket and balloon was three times as rapid as with blanket alone, 2) the warming time was halved by the use of a gastric balloon in addition to the blankets, 3) histologic studies of the gastric mucosa of dogs submitted to extreme temperatures (3°C to 65°C) for prolonged periods showed no damage to the gastric structure

RESULTS

To date we have induced hypothermia in 13 patients with blankets and gastric balloon The average rate of rectal temperature change in these patients is compared to that of a group of 11 patients whose age and somatic characteristics were similar to the first group This second group was selected from a number of cardiac surgery patients who were cooled by blanket alone Only 4 of the patients, however, were rewarmed by blankets These two groups will be hereafter referred to as A and B respectively

Group A consisted of 10 men and 3 women whose ages ranged from 27 to 65 years with an average of 41 Their mean weight was 150 pounds with a spread of 107 to 200 Eight of the patients had neurologic diseases requiring craniotomies in 7 instances and 1 internal carotid exploration Of the 5 remaining patients in this group, 4 underwent intracardiac surgery and 1 a thoracic aorta aneurysm repair One neurologic and 1 cardiac patient in this group expired on the operating table prior to rewarming from causes unrelated to hypothermia

In Group B 8 of the 11 patients were men and 3 were women Included in this group was a neurological patient who in separate operations was cooled and warmed by each technique The average age and weight of this group was 37 years and 150 pounds with a distribution similar to that in Group A The average cooling and warming blanket temperature in Group B was identical with that in Group A, i e —2°C and 46°C

In all instances hyperventilation by a mechanical respirator with a gas mixture of 95% O_2 and 5% CO_2 was used to maintain the blood pH within normal limits[4]

The comparative rates of rectal temperature change in each of the two groups is shown in Figures 2 and 3 Cooling Group A progressed at an average rate of 1°C every 19 0 minutes as opposed to 1°C every 31 1 minutes in Group B The difference in warming rates was likewise clear from the comparison of 29 5 minutes for 1°C change in Group A with 11 1 minutes for each degree centigrade in Group B

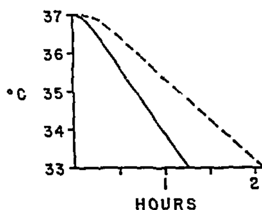


Fig 2 Average rate of rectal temperature change in 13 patients during cooling with blanket and gastric balloon (solid line) and in 11 patients with blanket alone (dashed line)

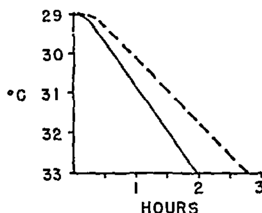


Fig 3 Average rate of rectal temperature change in 11 patients during warming with blanket and gastric balloon (solid line) and in 4 patients with blanket alone (dashed line)

The distribution of temperature change rates according to weight for the patients in Group A is seen in Figures 4 and 5

The remaining data are concerned with Group A only as it would seem superfluous to review the technique of blanket hypothermia here

Actual cooling of these patients covered an average range of 4 1°C the maximum being 6 3°C and the minimum 2 2°C This variation is attributed to two factors The first is the variability of preinduction body temperature which was from 35 0°C to 37 8°C The second factor is that temperature level required for the particular type of surgical procedure being performed Active cooling was stopped when the temperature reached an average of 32 9°C This figure also varied with the beginning temperature and the level desired

Individual variation in the amount of the drift was from 1 4°C to 5 6°C with an average of 3 6°C Drift was seldom a problem with this technique

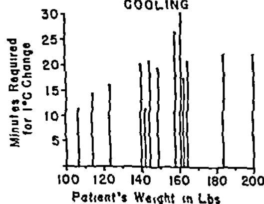


Fig 4 Distribution of the rates of rectal temperature change according to body weight in 13 patients cooled with intra gastric balloon and refrigerated blanket

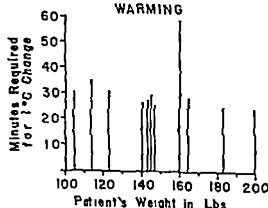


Fig 5 Distribution of the rates of rectal temperature change according to body weight in 11 patients warmed with intra gastric balloon and external blanket

since it generally followed the same rate of temperature drop as that seen during actual cooling This enabled accurate predictions of the time at which intragastric rewarming could be started to arrest the drift maintain the temperature at a desired level, or to rewarm the patient

In the one instance of thoracic aorta aneurysm repair which allowed limited contact between body and blanket the temperature was lowered maintained at 31 6°C ± 0 3°C for 3 hours and then raised to a safe level by means of the gastric device and a single lower blanket

The 11 patients that were rewarmed reached an average minimum temperature of 29 3°C covering a range of 27 0°C to 31 1°C These patients were actively warmed to approximately 34°C This level was attained in time to permit the patient to be safely transferred to a recovery room on completion of surgery Cessation of warming at this level was found to decrease the incidence of pyrexia in the immediate recovery period The one instance of post hypothermic shock or hypotension which occurred was believed due to the presurgical condition of a ruptured intracranial aneurysm

Changes in pulse rate, blood pressure, and electrocardiography of the 11 survivors were compatible with those found in hypothermia induced at a moderate rate

The first 3 patients cooled by intragastric balloon were subjected to pepsin and hydrochloric acid titers and no changes were detected

In no instance was there any postoperative evidence of esophagitis gastritis, mediastinitis, or acute gastric dilatation

Seven patients have been contacted 6 months to 1 year postsurgery and have no gastrointestinal complaints which were not present before surgery nor exacerbation of a preexisting condition

DISCUSSION

While our work with the intragastric balloon has been an investigation of its use in general hypothermia it has a number of further possible applications in local hypothermia

One recent development has been reported by Wangensteen and associates [5] They have utilized a gastric balloon as a means of controlling massive hematemesis and depressing secretory and digestive activity

SUMMARY

1 An intragastric balloon has been used in combination with thermic blankets to produce hypothermia in 13 patients

2 This technique produced more rapid body temperature change and enabled more accurate temperature control than that obtained when blankets alone were used

3 The gastric balloon was especially useful in surgery affording limited contact between body and blanket

4 The gastric apparatus has proved safe efficient and relatively easy to operate

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during infancy, without exception, pulmonary artery pressures on the se catheterization were lower than those recorded on the first examin These 9 include 3 of the 6 infants in congestive heart failure all of w responded well to digitalization and antibiotics and all of whom sh progressive clinical

Of the 29 childre

years of age when first catheterized and 10 were between 2 and 6 years ol The pulmonary artery systolic pressures at the first catheterization wer der 30 mm Hg in 3 patients, between 30 and 60 mm Hg in 12 patients over 60 mm Hg in 14 patients Of the 15 patients with pulmonary a systolic pressure less than 60 mm Hg at the first catheterization a l pulmonary artery pressure was recorded at the second catheterization in In the one exception in this group, the pulmonary artery systolic pres was recorded as 33 mm Hg on the first catheterization and 4 years later found to be 38 mm Hg

Fourteen children were found to have pulmonary artery systolic press greater than 60 mm Hg at the first catheterization In 3 of these 14 child significantly lower pulmonary artery pressures were recorded at the sec catheterization Pressures remained in the same range in 8 children showed a rise in 3 children Two of the children demonstrating a rise pulmonary systolic pressures on serial catheterizations were operated up One of the 2 operated upon was found to have an incomplete transpositio the great vessels, with the pulmonary artery and the aorta arising from right ventricle in the presence of a large ventricular septal defect Pulmon artery pressures in the second child operated upon were 77/35 at 3 year age and 92/57 at 6 years of age The defect in this child was closed successfu demonstrating at least that irreversible pulmonary artery changes did not t place over a 3 year period The third child showing a rise in pulmonary art pressures was catheterized at 6 and 10 years of age having pulmonary art pressures of 77/47 and 103/66 respectively The predominant shunts in t child were left to right at both examinations with no evidence of progress reversal of blood flow through the defect

Of the children catheterized once or followed by clinical examination on many show the effects of long standing pulmonary artery hypertension large left to-right shunts with repeated upper respiratory infections and tardation of growth and nutrition However, there has not been a single stance of reversal of flow demonstrated in followups from a few months 10 years In the relatively few patients encountered with ventricular sep defects and right to left shunts there is reason to suspect that this degree pulmonary arteriolar resistance has been present since early infancy, in mo if not all cases Ten of the children followed clinically were infants in seve congestive heart failure when first seen All of these 10 infants respond well to digitalization and antibiotics There were no deaths among these l and all showed progressive improvement in clinical symptoms after the fi year of life, without evidence of progressive pulmonary arteriolar resistanc This is in contrast to the reports by personal communication of several grou operating upon these infants in which the operative mortality ranged fro 28% to 100%

In addition to the operative mortality which certainly will improve wit more experience, a second deterrent to operation for small defects at th

present time must be brought out. Of the 16 children surviving operation, at least 5 have clinical or catheterization evidences that the defects have either reopened or were inadequately closed initially We do not know the experiences of other groups in regard to this problem, but the fact that some surgeons are changing from direct suture of the defects to the placement of patches, while others are changing from patches to direct suture leads us to suspect that others may be having the same difficulty. It is of further interest that those defects which still appear to have some residual shunt have in most instances been those of moderate size and pressures, while our best results have been in the children with large defects, high pulmonary artery pressures, and severe symptoms

SUMMARY AND CONCLUSIONS

1 Three hundred and fifty children with ventricular septal defects have been studied over a period of 10 years in an effort to determine the natural course of the disease, particularly in relation to the indications for surgical repair One hundred and ten of these children have been subjected to cardiac catheterization, 29 of them on two or more occasions at intervals of 1 to 7 years

2 Three of the children undergoing serial studies were found to have increased pulmonary artery pressures at the second catheterization All of the remaining children studied in this manner showed no change or showed a fall in pulmonary artery pressures with increasing age

3 No child in the entire group has developed a reversal of flow through a ventricular septal defect due to progressive pulmonary arteriolar resistance.

4 Of the 16 infants in congestive heart failure during the first year of life, not one has died, not one has developed evidences of increasing pulmonary arteriolar resistance, and all have shown progressive improvement in symptoms after the first year of life

5 It is our opinion, based upon the findings stated above, that only those children with large ventricular septal defects and symptoms associated with pulmonary hypertension and large left-to-right shunts should be operated upon at the present time The children with smaller defects and no symptoms may be followed without fear of their becoming inoperable, until such a time as technical improvements can insure a lower mortality and permanent closure of the defect It is doubtful at the present time that the risk of operation is ever justified in a child under one year of age.

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EXPERIMENTAL PRODUCTION OF CARDIOVASCULAR ANOMALIES IN DOGS USING TRYPAN BLUE *

J Harold Conn and James D Hardy

An extremely valuable method of studying disease processes is to reproduce them in experimental animals One of the difficulties in evaluating cardiovascular diagnostic and surgical procedures in the laboratory is that common cardiovascular anomalies have not been reproduced satisfactorily If various types of cardiac septal defects pulmonary outflow tract and great vessel anomalies could be quantitatively produced in an animal large enough for diagnostic and operative procedures a significant advance would be made

It has been conclusively shown by Ingalls[1] and others that congenital defects are not due to chance combinations of genes alone but that stressful events occurring to the gravid mother at critical periods in her pregnancy may cause cleft palate open eye anencephaly Mongolism and various cardiovascular anomalies

Cardiovascular anomalies have been produced in rat and mouse fetuses by subjecting the gravid female to x radiation vitamin D deficiency hypoxia vitamin A deficiency and folic acid deficiency Fox and Goss[2] recently produced a disproportionately large number of cardiovascular defects in rat fetuses by injecting trypan blue into the pregnant female rats Richman Thomas and Konikov[3] repeated this experiment to determine if the rat fetuses with cardiovascular anomalies could survive after birth Surprisingly of the 50 rat offspring selected at random in this study 27 had major cardiovascular defects of which 17 were interventricular septal defects 7 inter atrial septal defects 5 patent ducti and 3 stenosis or atresia of the pulmonary outlet A majority of these rats survived and grew normally for one month before they were sacrificed

The purpose of the following experiment was to determine if similar results could be obtained in an animal large enough for diagnostic and operative study

METHOD

Twenty seven female mongrel dogs were selected for this experiment Dogs were originally injected with 1 cc of 1% aqueous solution of trypan blue per pound of body weight on the tenth day of pregnancy However as 4 of these animals spontaneously aborted this was thought to be too great an amount of dye Consequently subsequent animals received 0 5 cc of dye per pound of body weight The tenth day of pregnancy was selected as the beginning of cardiovascular differentiation in the dog According to Ingalls the time of differentiation of the system to be deviated is the most appropriate time to give the stressful stimulus to the gravid animal

RESULTS

Twenty six pregnant dogs gave birth to 106 puppies in 29 litters Three dogs each had 2 litters Seventeen puppies were born dead or died within 3 days following birth All other puppies were sacrificed between 1 and 3 months Heart and great vessels were removed from all puppies and examined

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carefully for anomalies Only 8 cardiovascular defects were found patent ductus arteriosus 3, interventricular septal defect 2 interatrial septal defect, 2 single coronary ostium 1 One patent ductus occurred in the group that was born dead or died within 3 days All other anomalies occurred in the dogs sacrificed between 1 and 3 months of birth No external abnormalities were noted in any of the puppies

DISCUSSION

Although the number of cardiovascular anomalies found in this study may be significant statistically (we are not aware of any other study of congenital anomalies in the dog) it is certainly not a large enough yield to be practical for laboratory use

Of all the diazo dyes tested by Wilson[4] for teratogenic properties only trypan blue gave a high yield of cardiovascular anomalies Its mode of action upon the fetus is unknown It is accumulated in the reticuloendothelial system and renal tubules of the mother and may exert an indirect effect on the fetus by altering the maternal physiology The dye apparently does not cross the placenta, as it has not been demonstrated in the tissues of either the placenta or the fetus The time of administration of the dye may not be critical, as various fetal anomalies have been produced by mothers who received the dye prior to as well as during pregnancy Possibly the effect of the dye does not reach its height for some time after administration Two of the cardiac anomalies in this experiment occurred in puppies from mothers which had received the dye in successive pregnancies Baird *et al* demonstrated that folic acid deficiency produced cardiovascular defects in fetal rats primarily of the aortic arch as opposed to the greater number of cardiac anomalies found by Fox and Goss, using trypan blue The chemistry of trypan blue does not suggest that it is a metabolic antagonist to any of the vitamins[3]



Fig 1 Patent ductus arteriosus



Fig 2 Interatrial septal defect



Fig 3 Interventricular septal defect

SUMMARY

A successful method of producing cardiovascular anomalies in the rat has been evaluated in the dog, an animal more suitable for experimental diagnostic and surgical procedures Only 8 cardiovascular anomalies were produced in 106 puppies Although this may be significant from an expected deviation standpoint, it is not practical for laboratory use

This is a preliminary report, and further work will be done, varying the timing of the stress and combining other teratogenic agents

A most promising observation is that two of the cardiovascular defects



Fig 4 Single coronary ostium

occurred in puppies from subsequent litters of reinjected mothers This may mean that a longer period of time is necessary to sufficiently alter the maternal physiology to produce defects in its offspring

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CATHETERIZATION OF THE LEFT HEART THROUGH THE INTERATRIAL SEPTUM A NEW TECHNIQUE AND ITS EXPERIMENTAL EVALUATION *

JOHN ROSS JR

Left atrial puncture by the transbronchial[1, 4, 5] and percutaneous[3, 6] methods has had widespread clinical and physiologic application However both these approaches to the left heart have been found to have limitations in certain clinical situations The possibility that the intact interatrial septum might be crossed in the course of right heart catheterization was first suggested by Dr E del Campo as he was observing a catheterization study at the

* From the Clinic of Surgery National Heart Institute Bethesda Md

National Heart Institute A method has been devised in which the interatrial septum is punctured with a retractable needle passed through a cardiac catheter The details of this technique and its experimental application are described in the present communication

METHOD

Thirty seven adult mongrel dogs were studied All the animals were anesthetized with sodium pentothal Statham pressure transducers and a direct writing oscillograph were used for pressure measurements Electrocardiograms were monitored continuously

Left atrial puncture is accomplished with a long flexible needle passed through a Cournand catheter Stainless steel needles of two sizes are used one 19 gauge, the other 18 gauge thin wall † The needles are 61 cm in length curved at their distal ends, and have metal indicator handles attached to the needle hubs for ease in manipulation (Fig 1) The smaller needle passes through the lumen of a #8 Cournand catheter, while a #9 catheter is used with the larger needle The Cournand catheters are shortened to allow the needle tips to protrude 1 5 cm when fully inserted A small plastic catheter 80 cm long and 039 in in diameter,†† may be passed through the larger needle for catheterization of the left ventricle and aorta

The dog is placed on its left side on a fluoroscopy table with the right hind leg externally rotated The Cournand catheter is introduced through the right femoral vein into the right ventricle It is then withdrawn into the right atrium where a blood sample is obtained Under fluoroscopic control the

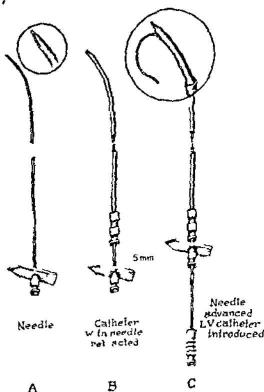


Fig 1 Design and details of the #18 gauge needle Cournand catheter and small plastic catheter

† Made by the Superior Tubing Co Norristown Pa

†† Vinyl compound tubing #442 T manufactured by the Becton Dickinson Co Rutherford N J

catheter tip is positioned at the junction of the inferior vena cava and right atrium The catheter hub is then disassembled and the needle is inserted into the catheter The hub end of the needle is rotated by an assistant in the sequence shown (Fig 2) while the operator advances the needle until its point lies just inside the distal end of the catheter The catheter tip is next directed posteriorly and somewhat medially until resistance is encountered approximately 1 cm above the mouth of the inferior cava in the region of the fossa ovalis (Fig 3A) It is then held stationary in this position while the needle is pushed forward through the septum into the left atrium (Fig 3B) A change in resistance is usually felt as puncture occurs The catheter and needle should then be withdrawn slightly until blood is obtained Correct placement of the needle is verified by visual comparison of the blood sample and pressure tracing with those previously obtained from the right atrium Occasionally an unsuccessful first attempt requires that the puncture be re peated at a slightly higher level in the atrium where the catheter tip may be lodged beneath the annulus ovalis

Angiocardiography with left atrial injection may be performed through the #18 gauge needle After the administration of a small test dose of 70% urokon 6 to 8 cc of dye are injected manually as serial x rays are exposed Use of the #18 gauge needle also permits passage of the small plastic catheter described above which may be manipulated across the mitral valve into the left ventricle When free forward passage of the catheter is obtained the needle point is withdrawn slightly within the Cournand catheter which re tains its original position against the septum Further manipulation of the left heart catheter may then be carried out

RESULTS

Transeptal puncture of the left atrium was performed in 37 dogs Twenty six of these were normal animals 10 had surgically produced chronic mitral insufficiency and one had a congenital ventricular septal defect

The #19 gauge needle was employed in 18 of the normal animals and in *all the dogs with mitral insufficiency* Left and right atrial pressure pulse contours were characteristically different *in all instances* [7] One animal showed atrial coupling following puncture although spontaneous reversion to sinus

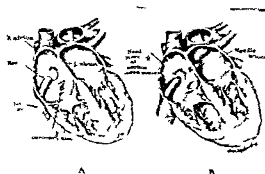


Fig 2 Technique for inserting needle *into* the Cournand catheter The needle hub is rotated clockwise after passing the bifurcation of the inferior vena cava

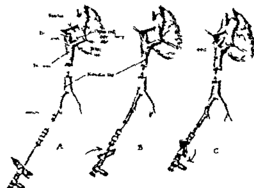


Fig 3 Position of needle tip before (A) and after (B) puncture of the interatrial septum

rhythm occurred almost immediately During right heart catheterization myocardial irritability was prominent in some of the dogs having mitral insufficiency, and at the time of septal puncture transient bigeminal rhythm occurred in three of these animals A sinus mechanism returned, however following removal of the needle

In fourteen dogs the #18 gauge needle was used Left atrial angiocardiograms were performed in nine of these animals

Two dogs died the night following the procedure Both of these animals had received two left atrial urokon injections totaling 20 cc One had a congenital ventricular septal defect with marked pulmonary hypertension and exhibited gross pulmonary edema at postmortem examination Autopsy of the second animal failed to reveal a cause of death

Twelve dogs were operated upon at intervals of from two weeks to four months following septal puncture with the #19 gauge needle The right atrium was opened, and a careful inspection of the interatrial septum carried out In 2 animals, small amounts of oxygenated blood could be forced through a tiny hole in the septum by manual compression of the left atrium One of these dogs was 7½ weeks, the other 3½ months postcatheterization

COMMENT

The present technique employs the venous system for left heart catheterization and thereby allows a single approach for both left and right heart studies In the laboratory, the method described has proved to be practical and safe Both of the deaths in the experimental series followed the injection of large doses of urokon, the increased risk associated with left heart catheterization combined with left atrial selective angiocardiography is well documented[6] Accidental perforation of the aorta or left ventricle was not encountered In man the interatrial septum is larger and the mediastinum less mobile than in dogs These factors should facilitate entry into the left atrium and make left ventricular catheterization less difficult. The persistence of the needle hole in 2 animals is somewhat puzzling, although it seems unlikely that a significant interatrial shunt was present

Left heart catheterization by the transeptal route has several possible clinical applications In infants and small children left heart catheterization may be difficult by present methods In such patients, left sided dye dilution studies or angiocardiography may be extremely helpful in the assessment of congenital deformities In addition it would be useful to have an alternative route available for the small group of adult patients in whom attempts at transbronchial or percutaneous left heart catheterization are unsuccessful Preliminary studies in autopsy specimens indicate that with minor modifications the technique described is applicable

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THE EFFECT OF L-NOREPINEPHRINE ON CARDIAC OUTPUT AND HEPATIC BLOOD FLOW IN EXPERIMENTAL ACUTE HEMORRHAGIC HYPOTENSION *

BERTRAM A. PORTIN, MALCOLM B. LESCH, ROGER E. G. MESMER AND WORTHINGTON G. SCHENK, JR.

Previous experimental studies done in our laboratory[9] have shown the effect of acute massive hemorrhage on portal blood flow. The present study was undertaken in an effort to determine the effect of l-norepinephrine on hepatic blood flow, peripheral blood flow and cardiac output in an acute hemorrhagic state. Such data could also be expected to shed some light on the question of the existence of a phenomenon of preferential redistribution of blood to the various crucial vascular beds during certain unphysiologic states.

METHOD

A series of anesthetized dogs was studied before and after acute hemorrhage and during and after l-norepinephrine infusion. Flow measurements in the ascending aorta, portal vein, hepatic artery and femoral artery were made using the electromagnetic flowmeter as described by Spencer, Denison and Green.

RESULTS

Effect of Hemorrhage. (Figure 1, column 1 and 2) *Portal flow.* In 6 experiments sudden massive hemorrhage resulted in a decrease of portal vein blood flow ranging between 7 and 77% with a mean decrease in flow of 40%. *Hepatic artery.* Similarly, hemorrhage resulted in a decrease in hepatic artery flow in all 6 instances. The range was 35% to 96% and the mean decrease was 52%. *Ascending aorta.* In all 6 experiments a decrease in cardiac output (left ventricular output minus coronary artery flow) was noted. The range was 27 to 50% with a mean decrease of 38%. *Femoral Artery.* In 5 experiments in which this parameter was measured, flow decreased from 20% to 70% with a mean decrease of 50%.

Effect of L-Norepinephrine. (Figure 1, column 2 and 3) *Portal flow.* In 5 of 6 experiments there was a definite increase in portal flow during the l-norepinephrine infusion phase of this study. The increase ranged from 8% to 200%. In 1 experiment a 30% decrease in portal flow was noted. The mean increase was 47%. *Hepatic artery.* In all 6 experiments there was a marked rise in hepatic artery blood flow, the average increase 66%. *Ascending aorta.* Flow measurements increased in this vessel in all cases, ranging from 7 to 203%. The mean increase was 75%. *Femoral Artery.* The most marked increase in blood flow during l-norepinephrine infusion occurred in this peripheral vessel. The average increase was 190%. Calculation of cardiac rate changes show a mean rise of only 10% when comparing values after hemorrhage with those noted during l-norepinephrine infusion.

Effect of L-Norepinephrine Withdrawal. (Figure 1, column 3 and 4) *Portal flow.* In 5 experiments 4 animals experienced a decrease in portal flow after l-norepinephrine withdrawal. In 1 experiment, there was a 30% increase of

* From the Department of Surgery, University of Buffalo School of Medicine and the Edward J. Meyer Memorial Hospital, Buffalo, New York. Supported in part by a grant from the United States Public Health (H-3118).

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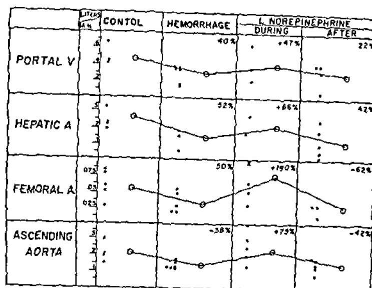


Fig 1 Changes in Mean Blood Flow

flow The mean decrease was 22% *Hepatic artery* In 4 of 5 animals a de crease in blood flow was noted The dog found to have an increase in portal flow had a 6% increase in hepatic artery flow also, the mean flow drop was 42% *Ascending aorta* In 5 experiments, all animals had significant decreases in cardiac output The range was 20 to 52% with a mean decrease of 42% *Femoral artery* In the 3 animals with measured femoral artery flows studied during this stage, the decreases noted were 42%, 64%, and 80% with a mean decrease of 62%

DISCUSSION

The preparation used in this experiment was designed to closely approxi mate a clinical situation based on trauma (thoracotomy and laparotomy) with acute blood loss (a single rapid hemorrhage rather than a protracted additive one) Other studies[3 5] have been done utilizing a modification of the method of Wiggers[10] which provides for continuous decrements and in crements of blood in an effort to maintain a desired level of hypotension We have felt that this type of preparation does not allow homeostatic mechanisms to operate and, therefore less closely approaches a clinical situation

The liver has become of increasing importance in the study of the shock state Earlier work from this laboratory as well as from others has demon strated a significant decrease in liver blood flow after hemorrhage There does not appear to be this unanimity with regard to the effects of l norepinephrine on the already depleted hepatic flow Both Gilmore[6] and Frank *et al*[3] have concluded that hepatic blood flow is not augmented under these conditions The results of this study do not support their conclusions

Levy and Brind[8] have demonstrated an augmented cardiac output during l norepinephrine infusion Gilmore *et al* showed a similar increase in early hypovolemia but were unable to maintain the increase during later stages while using a protracted bleeding technique The results obtained in this experiment show a definite rise in cardiac output during l norepinephrine infusion

Frank *et al*[3] attributes to norepinephrine the action of a preferential re distribution upon regional blood flow in hemorrhagic shock He showed an increase in adrenal, coronary, and cerebral blood flows and a decrease in hepatic and renal flows after l norepinephrine infusion in hypovolemic dogs

From the results presented we cannot substantiate a redistribution phenomenon. Comparison of flow rates after hemorrhage with those after cessation of l-norepinephrine show no significant variation. Whether l-norepinephrine inhibited homeostatic mechanisms in an effort to increase flow after hemorrhage or prevented further decreases in flow is a matter of speculation.

An interesting facet of this study was an evaluation of the relative importance of increased cardiac rate versus increased stroke volume in the light of the increased cardiac output noted after institution of the l-norepinephrine infusion. Cardiac output was noted to have risen 75%. Calculations of pulse rate changes after hemorrhage and after l-norepinephrine revealed a mean rise of 10%. Therefore, it would seem that a considerable portion of the increased cardiac output must have been due to an increase in stroke volume. It is not known whether this increase is due to an increased left ventricular filling and/or emptying.

CONCLUSIONS

1. In experimental hemorrhagic hypotension in dogs l-norepinephrine infusion elicits an increase in portal vein, hepatic artery, femoral artery and ascending aorta blood flow.

2. There does not appear to be a preferential redistribution of blood to the visceral vascular beds measured in this study.

3. The increased cardiac output found during l-norepinephrine infusion is more a function of an increase in stroke volume than an increase in cardiac rate.

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EXPERIMENTAL PRODUCTION OF ARTERIOSCLEROSIS IN THE DOG *

NORMAN W CRISP, JR, HARLAN D ROOT, CONRAD B JENSON, KAMIL IMAMOGLU AND EARL G YONEHIRO

Arteriosclerosis has been easily produced in the smaller laboratory animal such as the rabbit, guinea pig, and fowl, usually through the administration of cholesterol,[1 2 3] but these animals are unsuitable for investigative and therapeutic *surgical procedures* for myocardial revascularization The production of experimental arteriosclerosis in the dog has been difficult and time consuming, and successful only through the use of thiouracil and cholesterol[4 5] By this means the objections of Duff[6] and others to the comparison of the anatomical distribution and to the progression and maturation *of the experimentally* produced *arteriosclerotic lesions to those found in man* have been met

METHOD

Unselected, young (6 months to 3 years) male and female mongrel dogs weighing 10 to 15 kg, were subjected to total surgical thyroidectomy with careful preservation of the parathyroid glands After a convalescent period of 7 to 10 days, the dogs received vitamin D (Detalin †) 100,000 units orally each day, in addition to their regular diet of prepared dog biscuit (Kibbies) and canned dog food (Rival) No supplementary cholesterol or calcium was administered Control animals were divided into 2 groups (1) thyroidectomized animals not receiving vitamin D, and (2) normal, unoperated animals which received 100,000 units vitamin D daily Complete gross and microscopic *postmortem examinations were done on all animals with* the evaluation of the extent of arteriosclerotic disease being made by the same observer Serum cholesterol levels were determined weekly during the observation period after the method of Abell[7]

RESULTS

The thyroidectomy vitamin D group consisted of 16 dogs, 6 female and 10 male The mean control serum cholesterol level was 190 mg/100 cc with a range of 131 to 377 mg % The serum cholesterol determination during vitamin D therapy postoperatively averaged 217 mg % (Range 135 to 328 mg %) The difference in values is not statistically significant, and is within *the range of normal canine values expected* for cholesterol[4]

Nine of the 16 animals had severe gross arteriosclerosis upon postmortem examination, being graded III and IV plus with lesions in the sinuses of Valsalva, arch of the aorta, descending aorta, coronary and renal vessels and in the pulmonary artery Six of the 9 animals were males The remaining 7 dogs, *4 males and 3 females* had arteriosclerosis to a lesser degree, being graded I and II plus Thus the gross lesions of arteriosclerosis were present in 100% of the animals, representative examples of which are seen in Figures 1 and 2 Especially prominent is the proliferation about *the coronary* ostia and the aortic valve Microscopically, the characteristic findings included

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† Detalin Vitamin D 50 000 units per capsule Eli Lilly Co Indianapolis.

* From the Department of Surgery University of Minnesota Medical School Minneapolis

Supported by funds from an anonymous donor



Fig 1 Arch of the aorta in dog 1 Thyroidectomy—Vitamin D group after 25 days of therapy Verrucous proliferations from the aortic valve cusps in the middle right of the picture Also the glazed and roughened plaques in the aorta on the left



Fig 2 Aorta of Dog 2 after 90 days of therapy Many plaques are noted about the coronary ostia along the aorta and the carotid

medial deposition of amorphous material with calcification and intimal foam cells with degenerative and proliferative changes Figure 3 shows such intimal proliferation with calcification of the media The animals were followed for a period up to 225 days but severe lesions were usually produced within 90 days

The control thyroidectomized group consisted of 6 dogs whose mean control serum cholesterol levels did not differ significantly (mean 195 mg %, range 131 to 215 mg %) The postoperative levels were similar Five dogs without thyroidectomy receiving vitamin D had like values (control mean 140 mg % vitamin D mean 128 mg %) Complete pathological examination of tissue revealed no gross or microscopic evidence of arteriosclerosis In addition extensive experience in canine pathologic examinations has confirmed the rarity of canine arteriosclerosis occurring normally and spontaneously being confined to the very old dogs[8] The results are summarized in Table 1



Fig 3 Photomicrograph of lesions from dog 2 Note intimal proliferation in upper left with amorphous deposits medial destruction and calcification

DISCUSSION

Arteriosclerosis has been shown by many to be affected by hormonal factors The thyroid gland exerts a major effect upon the experimental production of arteriosclerosis[4, 6] as hypothyroidism accentuates and accelerates

Table 1 Summary of Results

DOG	SEX	CONTROL CHOLESTEROL mg%	DURATION OF THERAPY (days)	THERAPY CHOLESTEROL mg%	DEGREE OF ARTERIOSCLEROSIS
		THYROIDECTOMY VITAMIN D GROUP			
1	F	Pilot	25	Pilot	
2	M	study	27	study	1 plus
3	F	no	35	no	1 plus
4	F	values	42	values	1 plus
					4 plus
5	M	171	30	205	2 plus
6	F	225	48	215	3 plus
7	F	377	60	237	3 plus
8	M	154	84	183	3 plus
9	M	161	90	151	3 plus
10	M	—	90	135	3 plus
11	M	236	90	235	4 plus
12	F	—	150	328	1 plus
13	M	—	155	217	1 plus
14	M	131	210	225	3 plus
15	M	207	222	213	1 plus
16	M	166	225	287	3 plus
	9M 6F	190 Mean		217 Mean	
		THYROIDECTOMY GROUP			
17	M	131	20	161	0
18	M	89	130	154	0
19	F	215	30	244	0
20	F	195	14	—	0
21	M	131	14	—	0
22	M	241	100	540	0
		195 Mean		275 Mean	
		VITAMIN D GROUP			
23	M	106	100	151	0
24	M	84	35	124	0
25	F	222	30	218	0
26	M	159	100	160	0
27	M	112	85	84	0
		140 Mean		128 Mean	

the formation of lesions, while hyperthyroidism or the administration of desiccated thyroid exerts a protective and hindering influence[9] Some of these influences were confirmed in the present study It would be tempting to point out another hormonal factor As one can see from Table I the male dogs developed the more severe arteriosclerosis This, however, is probably not a valid conclusion from such a small series Further studies are proposed to

attempt to accelerate the pathological process, so that evaluation of therapeutic and corrective surgical procedures for myocardial revascularization can better be evaluated

SUMMARY

A predictable and rapid method of production of arteriosclerosis by means of surgical total thyroidectomy with preservation of the parathyroid glands and administration of vitamin D, 100,000 units per day orally with the animals on normal kennel rations is presented. Arteriosclerosis was produced in 100% of the animals, with severe lesions grossly visible in the aorta, pulmonary artery, and other vessels Microscopically, the lesions include medial deposition, calcification and intimal proliferation.

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HYPERCHOLESTEROLEMIA AND PULMONARY ARTERIOSCLEROSIS*

E M BERKAS, D J FERGUSON, AND R L VARCO

The relation between hypercholesterolemia and systemic atherosclerosis suggests that pulmonary arteriosclerosis may also be affected by changes in fat metabolism Atheromatous plaques are found in the main pulmonary arteries of older persons but seem to constitute no important part of pulmonary vascular thickening associated with pulmonary hypertension occurring in congenital heart disease[1] In the experiment to be described, the effect of hypercholesterolemia on pulmonary arteriosclerosis was tested in dogs in which pulmonary hypertension was produced by means of a systemic artery to pulmonary artery shunt

METHOD

In 7 young dogs weighing 8 to 10 kg, the left brachial artery was anastomosed to the distal end of the divided lower branch of the left pulmonary artery The left upper lobe was removed Pressure in the left pulmonary

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* From the Department of Surgery, University of Minnesota and the Veterans Administration Hospital Minneapolis. Supported by a research grant from the National Institutes of Health, Public Health Service, project H 1960

artery was initially elevated to mean values ranging from 25 to 45 mm Hg At a separate operation the thyroid gland was removed as completely as possible The animals received the usual ration of commercial dog food and water, supplemented by 5 gm of cholesterol and 40 ml of cottonseed oil 6 times a week Serum cholesterol was measured before operation when it ranged between 110 and 205 mg % and approximately twice a month there after Lung biopsies were obtained at intervals at which time the shunt pressure was again measured The sections were stained with Verhoeff s and Van Gieson s stain and interpreted by a system previously described[2] Pulmonary and systemic vessels were sectioned after freezing and stained for fat with Sudan IV A number of dogs failed to develop pulmonary vascular lesions or hypercholesterolemia and were excluded from the study

RESULTS

Gross postmortem examination of each of the 7 dogs studied at intervals of 46 to 104 weeks after operation disclosed the presence of atheroma in the aorta and in the coronary hepatic mesenteric renal phrenic iliac and uterine arteries The presence of sudanophile material in the lesions was confirmed by histologic study Serum cholesterol levels ranged from 514 to 1150 mg % before sacrifice Three dogs had severe (grade 3) pulmonary arterial changes[2] consisting of proliferative intimal thickening amounting to obliteration of the lumen in some vessels medial hypertrophy and collagenous widening of the adventitia The other 4 dogs showed less severe lesions mainly medial hypertrophy of the small pulmonary arteries

In one dog the left pulmonary artery pressure eventually was the same as that in the aorta (120 mm Hg mean) The left brachial artery showed atheromata but none were present distal to the anastomosis in the pulmonary artery Final mean pressures in the other dogs ranged from 30 to 58 mm Hg

Many sections of lung tissue from the 7 dogs showed only 2 vessels with slight traces of fat and the other vascular lesions were not distinguishable from those in dogs without hypercholesterolemia observed in previous studies of experimental pulmonary hypertension

DISCUSSION

The results of this experiment suggest that metabolic processes in the pulmonary arteries differ from those in systemic vessels in respect to cholesterol The hypotheses[3,4] that atheroma develops as a result of filtration of fat through a traumatized area of intima or as the result of small hemorrhages into damaged areas from which almost everything but the fat is reabsorbed do not seem adequate to explain the absence of atheroma in severely damaged pulmonary vessels In the early stages of the pulmonary lesions small hemorrhages are common and the severity of the trauma to the small arteries is measured by the fact that the reparative process often obliterates the lumen Nevertheless the pulmonary vessels remain unaffected by hypercholesterolemia while the systemic arteries not subjected to any unusual stress develop atheromatosis

CONCLUSION

Hypercholesterolemia in dogs of sufficient degree to produce systemic atheromatosis has no apparent effect on pulmonary arteriosclerotic lesions developing as a result of a systemic artery to pulmonary artery shunt

Atheromata do not occur in the pulmonary lesions even when they are severe enough to obliterate the lumen

REFERENCES

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AORTIC VALVE REPLACEMENT WITH A SUBCORONARY BALL VALVE *

W Sterling Edwards and Lloyd Smith

This is a progress report of a four year study on complete subcoronary replacement of the aortic valve in experimental animals

The stimulus for this project has been the encouraging research of Hufnagel[2] with his descending aortic ball valve Though an effective valve can be inserted quickly in the descending aorta this location unfortunately does not significantly reduce the degree of regurgitation at the diseased valve With present day techniques of extracorporeal circulation valves can be placed in the ascending aorta distal to the coronaries Two disadvantages to this position of the valve are immediately apparent. One of the major dynamic aberrations in aortic insufficiency is poor coronary blood flow since a large portion of normal coronary perfusion occurs in diastole when the aortic valve is closed Placement of the valve just distal to the coronary openings would siphon off an even greater portion of each systolic outflow

A second reason for seeking a subcoronary prosthesis in preference to one in the ascending aorta is the need for a complete valve replacement in cases of aortic stenosis The relatively poor results achieved by dilating calcified postrheumatic valvular stenosis will be improved only by excision of the diseased valve followed by the insertion of a prosthesis

In deciding which valvular principle to use we soon became wedded to the ball valve Flap wafer and spring valves all have two serious faults Stasis anywhere in the blood stream such as occurs behind flaps will often result in thrombosis This does not occur behind natural aortic valve cusps because of normal endothelium which is unwettable and quite resistant to thrombosis It will take at least several weeks for a nonwettable new lining to cover the surfaces of any prosthesis[3] During the healing stage of the neointima any stagnation of blood behind flaps will lead to a deposition of fibrin Only a ball valve can be made so that each heart beat completely washes all surfaces of the ball and inner surface of the prosthesis

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Gross postmortem examination of each of the 7 dogs studied at intervals of 46 to 104 weeks after operation, disclosed the presence of atheroma in the aorta, and in the coronary, hepatic, mesenteric, renal, phrenic, iliac and uterine arteries The presence of sudanophile material in the lesions was confirmed by histologic study Serum cholesterol levels ranged from 514 to 1150 mg % before sacrifice Three dogs had severe (grade 3) pulmonary arterial changes,[2] consisting of proliferative intimal thickening amounting to obliteration of the lumen in some vessels, medial hypertrophy, and collagenous widening of the adventitia The other 4 dogs showed less severe lesions mainly medial hypertrophy of the small pulmonary arteries

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The second and even more serious criticism of flaps and spring valves is the inevitable fatigue of any material plastic or metal exposed to body fluids which function as dilute acids No material now known can be expected to flex forty million times a year without serious breakage especially when immersed in a dilute acid Hufnagel s work has demonstrated the long term effectiveness of the intravascular ball valve without wear and with minimal problems of thrombosis

A number of workers including Hufnagel have considered the problem of a subcoronary ball valve but have apparently abandoned the idea because of mechanical difficulties of applying this principle in the limited space of the left ventricular outflow tract The major problem is how to insure an adequate channel around the ball during ventricular systole without introducing too much resistance In the descending aortic valve of Hufnagel this has been achieved by widening the lumen of the prosthesis at the proper point to allow adequate flow around the ball There are no space limitations in the descending aorta

To study precisely the anatomy of the aortic valve area in dogs latex rubber molds were made of the ascending aorta and sinuses of Valsalva (Fig 1) Measurements of the relative diameter of the aorta and sinus were made and the position of coronary orifices carefully noted From these measurements it was found that careful designing would allow the placement of a ball in the sinus area without introducing significant resistance during systole using the greater diameter of the sinuses in relation to the aorta above

The second major mechanical problem was fixation of the prosthesis with precise alignment of the coronary openings Figure 2 pictures various stages in our efforts to solve this problem All of the valves shown were machined from lucite with a silicone rubber coated nylon ball with one exception The valve B shown in Figure 2 utilized a free floating wafer After a few days thrombus uniformly occurred on top of the wafer in the area of stagnant flow This reinforced our opinion that the ball valve is the only practical principle These were inserted into the proper position through a longitudinal incision in the ascending aorta using extracorporeal circulation and potassium citrate arrest The aortic valve leaflets were excised in all animals External fixation was accomplished in valves A B and C by a silk ligature around the aorta or a multiple point nylon fixation ring (Hufnagel) The coronary openings in the valve were carefully placed under direct vision so that struts would not block the coronaries In approximately 50% of the animals with externally



Fig 1 Latex rubber mold of the aortic valve area in the dog

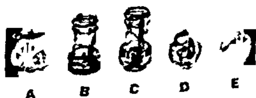


Fig 2 Various valves used in the aortic sinuses as described in the text

fixed valves the heart began to beat promptly after removing the aortic clamp and these animals recovered rapidly from anesthesia The other half suffered various cardiac disturbances chiefly ventricular fibrillation which could not be converted by electric shock or rearrest with potassium Autopsy showed that a coronary orifice was obstructed by a strut or by the rim of the basket in these animals and it became obvious that external fixation of the valve in the ascending aorta was not reliable in positioning the coronary openings properly no matter how large these openings were made Those dogs that survived the procedure lived from a few hours to 2 weeks and allowed an opportunity for pressure measurements proximal and distal to the valve These were most encouraging since there was little difference in systolic pressure in the ventricle and ascending aorta and pulse contour in the aorta distal to the valve was normal (Fig 3) with normal diastolic pressure

The cause of death in this group of animals was occasionally infection but principally thrombus formation in the valve A helpful technique was developed to determine the origin of thrombosis As soon as the animal began to show signs of failing whether hours or weeks after valve insertion it was heparinized and immediately sacrificed In this way postmortem clotting could not becloud the examination The major points of fibrin deposition were the junction of the struts with the distal fixation ring The projecting edges picked up fibrin and clots began which eventually blocked the lumen This method of fixation was therefore discarded because (a) one could not reliably position the coronary openings and (b) the fixation ring and struts presented projecting edges which trapped fibrin and led to thrombosis

The next effort at fixation was by direct suture of the body of the valve in the sinus area after excising the natural valve Valves of the design shown in Fig 2 D were based on the same principle except there were 2 or 3 shoulders to catch the valve and no external struts or fixation ring Placement of this valve was accomplished by two sutures inserted through the incision in the aorta one placed below each coronary and then through a hole in the rim of the valve and tied The anterior portion of the rim of the valve was fixed by additional sutures brought through holes in the solid plastic and then through the aorta to be tied externally After closing the aortic incision and releasing the aortic clamp these animals uniformly resumed a normal rhythm without the high incidence of fibrillation seen in the previous valves This was felt to be due to more reliable placement of the valve in relation to the coronary opening These animals died several hours to several days after

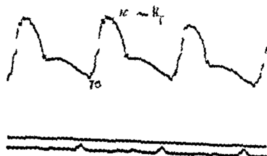


Fig 3 Pressure in the ascending aorta distal to a valve as shown in Fig 2 C. One week after insertion

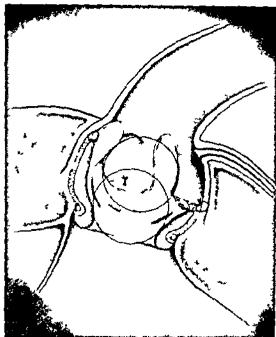


Fig 4 Position of the subcoronary ball valve in the sinuses of Valsalva



Fig 5 Method of fixation of the valve through a longitudinal incision in the aorta

operation The uniform cause of death was the tearing out of one or more sutures with acute angulation of the lumen of the valve followed by obstruction or insufficiency One valve was found far down the descending aorta after all its sutures had pulled out No matter how carefully the holes were rounded the sawing effect of the valve on the sutures either cut the sutures or pulled them out of the aortic wall

To overcome this problem a sleeve of synthetic cloth was wrapped around the outside of the body of the valve (Fig 2 E) Depressions were cut in the rim of the valve posteriorly one to fit below each coronary orifice A lip of fabric was allowed to extend 1 mm beyond the rim at the bottom of these coronary depressions (Fig 4) to hold a single suture placed as before below each opening The body of the valve was then fixed anteriorly by a suture across the lower end of the aortic incision which included the fabric around the valve (Fig 5) After the aortic incision was closed two additional sutures were taken one of each side through the aorta and into the fabric and tied externally This has produced very satisfactory fixation without the danger of threads tearing out since all sutures go through cloth and not plastic The rough surface of the plastic mesh has two other functions its frictional surface prevents any tendency to slide out of place and it fills out the space around the body of the valve making a snug fit in the aorta Valves of this type are extremely easy to insert and none has pulled loose Placement of the valve and complete closure of the aorta takes less than 15 minutes We feel that this valve has overcome the dynamic problem of resistance of the ball and fixation below the coronaries Sufficient time has not yet elapsed to determine if this type will be free of clotting problems Occasional problems of insertion at exactly the correct angle have arisen and tilting of the valve is sometimes troublesome Much more work must be done before a uniformly reliable ball valve is available

SUMMARY

Four years experience with efforts to develop a subcoronary ball valve have led us to the following conclusions

1 A really satisfactory valve should be able to replace the excised aortic valve and lie below the coronary orifices

2 The ball valve principle is the only one where material fatigue is not a problem and where the inside of the prosthesis and all surfaces of the ball are washed clean by every systolic pulse

3 It has been possible to design subcoronary ball valves which do not offer significant resistance to flow around the ball if the space in the coronary sinuses is economically used

4 Major problems have been methods of fixation of the valve without obstructing the coronary orifices

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GROSS AND MICROSCOPIC SEQUELAE IN EXPERIMENTALLY PRODUCED AND REPAIRED AORTIC ANEURYSMS *

Steven G Economou C Bruce Taylor and Edward J Beattie Jr

The increasing number of complications secondary to aortography[1, 2, 3] prompted this investigation on possible arterial wall damage by aortographic contrast solutions A plausible mechanism for such damage would be intramural injection and dissection of the highly irritating contrast solution

METHOD

The thoracic aorta of mongrel dogs was approached through a left thoracotomy incision and using a 27 gauge needle 1 cc of 70% acetrizoate (urokon) was injected intramurally The animals were sacrificed at intervals of from 3 to 30 weeks Gross aneurysms resulted (Fig 1) Microscopic examination of the excised aneurysms revealed intramural dissection hemorrhage and marked destruction of the media (Fig 2) The internal elastic membrane seemed to act as a barrier to the urokon and thus prevented it from injuring the subendothelial layers Significantly there was a lack of repair in aneurysms produced as long as 30 weeks previously This lethargic response to injury was in sharp contrast to earlier studies[4, 5, 6]



Fig 1 Saccular aneurysm measuring 2 by 2.5 cm in wall of thoracic aorta of dog sacrificed 5 months after injection of 1 cc of 70% acetrizoate (urokon) intramurally into media Aneurysms were observed as early as 3 weeks and as late as 30 weeks after intramural injections of urokon

* From the Departments of Surgery and Pathology Presbyterian St Luke's Hospital Chicago Supported by the Otho S A Sprague Memorial Institute the Chicago the Illinois and the American Heart Associations and the Life Insurance Medical Research Fund

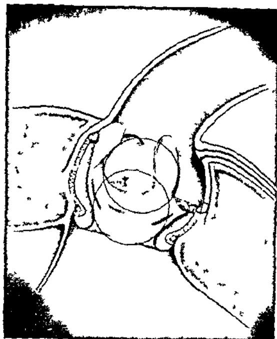


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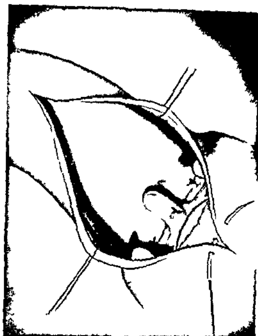


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Fig 2 Photomicrograph of typical early lesion (5 days after injection of 70% urokon showing intramural dissection hemorrhage and marked early destruction of the media The clear pale area surrounding the intramural dissection and hemorrhage (upper left) represents necrotic vascular tissue showing almost complete loss of dark staining nuclei



Fig 3 A saccular aneurysm is present at each end of the specimen They are in the wall of the thoracic aorta of a dog sacrificed 9 weeks after removing 60 to 70% of the media They showed no gross or microscopic evidence of repair The small central aneurysm resulted from an incomplete injection of 70% urokon in the media

It appeared that injury to the subendothelial layer of the aorta was the trigger mechanism to stimulation of the reparative process Therefore without subendothelial injury following the urokon injection repair was not initiated To further test this thesis we produced aneurysms by removing 60 to 70% of the media of thoracic aortas without injuring the remaining inner media and intimal layers Aneurysms developed immediately and persisted (Fig 3) Animals were sacrificed at intervals and on microscopic examination again there was no evidence of repair in aneurysms produced as long as 30 weeks previously Thoracotomy was performed a second time on a group of animals with aneurysms produced by medial stripping and one half of each aneurysm was frozen *in situ*[5, 6] for 1 minute so as to injure the subendothelium The animals were sacrificed biweekly Examination of these aneurysms revealed profuse subendothelial proliferation at the site of transmural freezing (Fig 4) In the adjoining area where no subendothelial injury had occurred there was no evidence of an attempt at repair



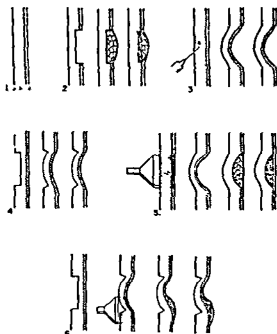
Fig 4 Photomicrograph illustrating subendothelial proliferation following transmural freezing at margin of aneurysm which had originally been produced by removing 60 to 70% of the media The full thickness of the aorta is shown at the right The cut edge of the outer media is centrally located and the thin aneurysmal wall is on the left Note the tuft of fibroelastic subendothelial tissue that has grown at the frozen margin of the aneurysm

SUMMARY AND CONCLUSIONS

As diagrammatically illustrated in Figure 5 when 70% acetrizoate (urokon) was injected intramurally in the thoracic aorta of dogs gross aneurysms resulted in most instances Aneurysms were also produced by stripping the media from the thoracic aorta of dogs When aneurysms produced by both of these techniques were examined as long as 30 weeks later there was no evidence of a reparative process

We were able to demonstrate that the stimulus to repair of such aneurysms was trauma (in this instance quick freezing) to the subendothelial layer The application of this technique in the repair of human arterial aneurysms should be studied further

Fig 5 Diagramatic presentation of reparative response of aorta following injury of various portions of its wall (1) Normal aortic wall a adventitia b media c internal elastic membrane and d endothelium (2) Removal of endothelium subendothelium internal elastic membrane and superficial media results in proliferation of multipotential subendothelial cells and formation of tuft of new fibroelastic tissue and near restoration of normal architecture and thickness (3) 70% acetrizoate (urokon) injection into media produces severe medial necrosis but spares internal elastic membrane subendothelium and endothelium No reparative response is present as late as 30 weeks after injury (4) Surgical removal of outer media also spares internal elastic membrane subendothelium and endothelium Here again there is no reparative response in the resultant aneurysm (5) Transmural local freezing of the aorta results in an aneurysm which is followed in a few weeks by proliferation of multipotential reparative cells from the site of subendothelial injury Within 5 to 6 weeks the aneurysms are repaired (6) Application of the same transmural freezing technique to aneurysms produced by medial stripping which otherwise showed no repair resulted in prompt subendothelial proliferation at the site of hypothermal injury



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COMPARISON OF FLAT AND CRIMPED DACRON TAFFETA ARTERIAL PROSTHESES OTHERWISE IDENTICAL *

ORMAND C JULIAN H H SU SAAD EL ISSA
ANDRE LIMA AND M LOPEZ BELIO

A method of weaving tubes and bifurcations of dacron in taffeta form for arterial substitution has previously been reported from this laboratory [1] The various clinical advantages of the wide choice of sizes available through this method have been pointed out in these publications These prostheses † are woven by a process termed duplicate weaving which results in the various tube and bifurcation shapes which have selvedge like edges but no internally visible or effective seams The strength and smoothness of the prostheses has been pointed out

One of the forms developed by this method consists of a tube 10 mm in expanded diameter having a thread count of 70 denier dacron of 200 by 180 per linear inch This tube was originally developed for the purpose of testing the fabric from the standpoint of tissue acceptance and strength in animals for later human implantations of the larger aortic bifurcations After such animal testing the material has been used extensively in clinical work The strength lack of tissue reaction and reliability of the fabric were found satisfactory in both The variety of sizes of bifurcation was found to be a distinct advantage in clinical replacement of the aortic bifurcation particularly for aneurysm

The advantages which could accrue from the use of crimped prostheses were realized from the work of Edwards [2] It was thought that the thinness of the taffeta woven prostheses would be maintained while the addition of crimping would technically improve the artificial vessels

Crimping in a relatively fine manner was easily accomplished with both the tubes and the bifurcations by threading them onto suitable metal mandrels which they fitted tightly compressing them into closely spaced folds and applying heat When cooled and removed from the mandrels the structures remained crimped through washing autoclaving and wetting with blood at the operating table All prostheses were found to have longitudinal elasticity and a strong tendency to remain expanded in round form Bending to greater than 90° and even twisting at the rate of one turn for each 8 in was accomplished without kinking

The advantage of this elasticity and ability to bend without kinking was immediately evident particularly in clinical work The ability to traverse joints at hip and knee level was judged to be valuable in the tube form while the greater ease of applying the grafts with end to side anastomoses was thought to be essential [3]

It remained to demonstrate in animals that the mechanical alteration of crimping or some chemical change in the dacron during heating did not alter the host reactions in an unfavorable manner Comparison was therefore sought between the original flat grafts and the new crimped prostheses

† Prostheses supplied by the Meadox Weaving Company Haledon N J

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Aided by grants from the Chicago Heart Association and the Ethel and Titus Hafla Cardiovascular Fund

METHOD

Seven to 10 cm lengths of the flat woven tubes and the crimped woven tubes were implanted in either the thoracic or abdominal aorta of mongrel dogs In the conservation of material about half of the implants were observed as separate experiments in the thoracic and abdominal aortas of the same dogs These were always placed at 2 operations staged 2 to 4 weeks apart In every instance the grafts were inserted in end to end fashion after resection of an equal or slightly shorter segment of aorta The anastomoses were made using 5 0 braided silk as an over and over continuous suture All operations were done under sterile conditions without the addition of heparin during the procedure The grafts were preclotted in the recipient s blood for 5 to 10 minutes before implantation

DISCUSSION

The initial observations were made at the time of implantation These concerned the ease of handling of the tubes the tendency to bleed after restoration of blood flow and the tendency to kink when discrepancies in length were inadvertently allowed to occur The comparison of these factors in use of the flat and crimped grafts was added to the analysis of the success rate of implantation

Later observations were made in the 2 types of graft after periods of from 3 to 6 months These consisted of aortogram gross examination with photograph of each graft externally and after opening and microscopic examination of a suitable number of the specimens

RESULTS

Initial Observations A striking difference was noted in favor of the crimped material in regard to the ease with which implantation was accomplished The difference in implantation arises from the round open end of the *crimped graft and the greater latitude in length permissible* because of its longitudinal elasticity These factors plus the greater speed with which they can be inserted resulted in the rate of success comparison recorded in the following table

Table 1

	FLAT DACRON	CRIMPED DACRON
Number of implants	24	36
Initial success	20	36
% initial success	83 3	100 0

Mild bleeding occurred through the preclotted fabric in a number of instances with each type of graft Severe bleeding was not observed The period of bleeding was brief in most instances It was not recorded as a comparison factor because of lack of severity

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COMPARISON OF FLAT AND CRIMPED DACRON TAFFETA ARTERIAL PROSTHESES OTHERWISE IDENTICAL *

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A method of weaving tubes and bifurcations of dacron in taffeta form for arterial substitution has previously been reported from this laboratory [1] The various clinical advantages of the wide choice of sizes available through this method have been pointed out in these publications These prostheses † are woven by a process termed 'duplicate weaving' which results in the various tube and bifurcation shapes which have selvedge like edges but no internally visible or effective seams The strength and smoothness of the prostheses has been pointed out

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METHOD

Seven to 10 cm. lengths of the flat woven tubes and the crimped woven tubes were implanted in either the thoracic or abdominal aorta of mongrel dogs. In the conservation of material about half of the implants were observed as separate experiments in the thoracic and abdominal aortas of the same dogs. These were always placed at 2 operations staged 2 to 4 weeks apart. In every instance the grafts were inserted in end-to-end fashion after resection of an equal or slightly shorter segment of aorta. The anastomoses were made using 5-0 braided silk as an over and over continuous suture. All operations were done under sterile conditions without the addition of heparin during the procedure. The grafts were preclotted in the recipient's blood for 5 to 10 minutes before implantation.

DISCUSSION

The initial observations were made at the time of implantation. These concerned the ease of handling of the tubes, the tendency to bleed after restoration of blood flow, and the tendency to kink when discrepancies in length were inadvertently allowed to occur. The comparison of these factors in use of the flat and crimped grafts was added to the analysis of the success rate of implantation.

Later observations were made in the 2 types of graft after periods of from 3 to 6 months. These consisted of aortogram, gross examination with photograph of each graft externally and after opening, and microscopic examination of a suitable number of the specimens.

RESULTS

Initial Observations. A striking difference was noted in favor of the crimped material in regard to the ease with which implantation was accomplished. The difference in implantation arises from the round, open end of the crimped graft and the greater latitude in length permissible because of its longitudinal elasticity. These factors, plus the greater speed with which they can be inserted, resulted in the rate of success comparison recorded in the following table.

Table 1

	FLAT DACRON	CRIMPED DACRON
Number of implants	24	36
Initial success	20	36
% initial success	83.3	100.0

Mild bleeding occurred through the preclotted fabric in a number of instances with each type of graft. Severe bleeding was not observed. The period of bleeding was brief in most instances. It was not recorded as a comparison factor because of lack of severity.

Among the factors affecting ease of handling was the tendency of the woven material to fray. The tubes formed of flat dacron required heat fusion of the ends in order to avoid troublesome threads in the line of anastomosis. Heat seal was continued for a time in the crimped tubes but was soon found to be entirely unnecessary and was abandoned.

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Late Observations Aortograms were done in each of the 52 animals visualizing the total of 56 grafts which had initially succeeded Study of the aortograms indicated that 3 of the initially successful 20 flat dacron implants had closed and that 2 of the initial 36 implants of crimped material had become obstructed Distinct kinking was frequently observed in the aortograms of implants of flat material There was no characteristic difference noted in the aortograms of flat implants 3 and 6 months after implantation Aortograms of the crimped grafts did not show kinking in any case although a kink was observed grossly in 1 of the 2 thrombosed grafts in this series The x ray at 3 months in 20 animals sacrificed at this period showed the pattern of the crimps in the edge of opaque media At 6 months the x rays of 8 animals with 14 patent grafts failed to show any remnant of this crimp pattern

Gross examination of the grafts *in situ* unopened showed no appreciable difference in the thickness or consistency of the fibrous tissue covering of flat or crimped tubes In general the fibrous envelope was thicker at 6 months than at 3 months Seven of the crimped dacron tubes in animals sacrificed at 3 months were enclosed in a fluid filled sac of fibrous tissue The fluid strongly suggested in appearance an old hematoma In every such case the graft was patent and did not in itself appear different from others In the 11 grafts examined after 6 months this encapsulated hematoma was present in 2 cases A similar hematoma was not encountered in any of the flat dacron implants

The rate and completeness of final lining of the grafts did not differ from flat to crimped structures Ten centimeters was the maximum length of graft implanted The relation of the new lining to the irregularities of the crimped internal surface was particularly recorded At 3 months significant covering had been deposited or had grown throughout the extent of 10 of 20 crimped grafts Of the remaining 10 there appeared to be no covering in 2 grafts one of which had closed with thrombosis an unknown time before sacrifice Eight showed incomplete lining developing from the anastomosed ends At 6 months all of the crimped grafts were lined and whereas the earlier lining had followed the contour of the crimps at this interval the depressions were filled and the more or less opaque lining was smooth Only 3 of 11 6 month crimped grafts failed to show this complete smooth lining

CONCLUSIONS

The comparative testing of the flat and crimped fabric tubes as replacement grafts in the aortas of dogs has resulted in observations distinctly in favor of the crimped product A higher initial success rate is attributable to the greatly improved ease of handling of the elastic and expanded crimped tube In animals the ability of the crimped tube to bend was not taken advantage of because no graft traversed an area subject to much bending The additional advantage of this bending without kinking has been repeatedly observed in the implantation in man of the same graft traversing the hip or knee joint or both

Old hematomata were observed surrounding crimped tube implants in 7 of 20 dogs studied after 3 months and in 2 of 11 grafts after 6 months This was not observed in any flat fabric implant The occurrence of this hematoma and the absence in flat grafts is unexplained

Tissue lining of the implants occurs at about the same rate and with the same thoroughness in the 2 types of graft The lining in the case of the

crimped material becomes thick enough in 6 months to fill up the relatively shallow crimps of this graft

SUMMARY

1 Arterial prostheses constructed of taffeta woven dacron cloth were tested as tubes with and without applying a crimping process

2 Ease of handling initial success and maintained patency was improved by the crimping process

3 A significant incidence of hematoma surrounding the grafts was observed 3 months after implantation of the crimped but not the flat tubes At 6 months the incidence was less The encapsulated hematomata did not affect graft function or the development of a lining The phenomenon is unexplained

REFERENCES

1 Julian O C Deterling R A Jr Bhonslay S Su H H Dye W S Lopez Belio M Surgery *41* 50 1957

2 Edwards W S Tapp J S Surgery *38* 61 1955

3 Julian O C Deterling R A Jr Bhonslay S Javid H Dye W S Lopez Belio M Grove W J Dacron Tube and Bifurcation Arterial Prostheses Produced to Specification II Continued Clinical Use and Addition of Crimped Tube Arch Surg *In press*

HEMODYNAMIC FACTORS IN ARTERIAL GRAFTING AN EXPERIMENTAL STUDY OF ANASTOMOTIC TYPES GRAFT SIZE AND GRAFT SURFACE CHARACTERISTICS *

D EMERICK SZILAGYI JOHN G WHITCOMB PETER WAIBEL AND WALTER SCHENKER

The various angioplastic techniques that have become popular during recent years in the replacement surgery of the peripheral blood vessels have often boldly modified many of the factors that determine the flow characteristics of the circulating blood The new vascular pathways created by the surgeon often differ from the host arteries (whose place they are meant to take) with respect to length diameter, configuration and surface texture These deviations have been particularly evident since the introduction of plastic arterial prostheses that present many types of manufacture and a wide variety of dimensions While the physiological artefacts created by the surgeon must have important bearing on blood flow and therefore on clinical results quantitative observations on this relationship have *not* been reported—indeed reliable observations of this type on the clinical level are extremely difficult if not impossible to gather Even in the experimental area published data pertinent to these problems have been scanty and have been obtained from studies on artificial models [1] It seemed to us therefore that an investigation of some of the hemodynamic problems posed by angioplastic surgical

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Late Observations Aortograms were done in each of the 52 animals visualizing the total of 56 grafts which had initially succeeded Study of the aortograms indicated that 3 of the initially successful 20 flat dacron implants had closed and that 2 of the initial 36 implants of crimped material had become obstructed Distinct kinking was frequently observed in the aortograms of implants of flat material There was no characteristic difference noted in the aortograms of flat implants 3 and 6 months after implantation Aortograms of the crimped grafts did not show kinking in any case although a kink was observed grossly in 1 of the 2 thrombosed grafts in this series The x ray at 3 months in 20 animals sacrificed at this period showed the pattern of the crimps in the edge of opaque media At 6 months the x rays of 8 animals with 14 patent grafts failed to show any remnant of this crimp pattern

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SUMMARY

1. Arterial prostheses constructed of taffeta woven dacron cloth were tested as tubes with and without applying a crimping process.

2. Ease of handling, initial success, and maintained patency was improved by the crimping process.

3. A significant incidence of hematoma surrounding the grafts was observed 3 months after implantation of the crimped but not the flat tubes. At 6 months the incidence was less. The encapsulated hematomata did not affect graft function or the development of a lining. The phenomenon is unexplained.

REFERENCES

1. Julian, O. C., Deterling, R. A., Jr., Bhonslay, S., Su, H. H., Dye, W. S., Lopez Belio, M.: Surgery, *41*: 50, 1957.
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3. Julian, O. C., Deterling, R. A., Jr., Bhonslay, S., Javid, H., Dye, W. S., Lopez Belio, M., Grove, W. J.: Dacron Tube and Bifurcation Arterial Prostheses Produced to Specification. II. Continued Clinical Use and Addition of Crimped Tube. Arch. Surg., *In press*.

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procedures would merit investigation in the animal laboratory under conditions in a measure approximating the milieu of the operation room

METHOD

The factors studied were 1) the type of anastomosis that is end to end compared to various types of end to side anastomoses 2) bypass grafting as compared to direct replacement 3) the diameter of the implant 4) the luminal surface characteristics of the implant In all 4 groups of experiments the basic plan was the same one common iliac artery of the animal was used as control and the other common iliac artery as the experimental vessel (that is to say the vascular substitute to be studied was inserted on the experimental side in a predetermined manner while the control side was either left intact or subjected to a mock operation) and the rate of volume flow of blood was determined simultaneously on both sides every effort being made to assure bilaterally identical physiological conditions except for the factor investigated

In dogs weighing 30 to 70 pounds the aortic bifurcation and iliac arterial systems were exposed through a long midline incision under nembutal anesthesia After both iliac arteries had been dissected from their beds on the experimental side the technical procedure to be studied was carried out For the measurement of blood flow the bubble flow principle was used as described by Dumke and Schmidt [3] Both the control and the segment of the experimental iliac artery distal to the area of surgical interference were connected by means of appropriate polyethylene tubing to separate bubble flow meters (in a constant temperature bath) 2 ml of air was injected into the afferent lead of each meter and the speed of the bubble propelled by the flowing blood through a glass coil of known capacity was accurately timed a simple calculation gave the amount of blood traversing the meter during unit time The injected air bubble was trapped on the efferent side of the meter before the blood was shunted back into the distal iliac arterial trunk By allowing the blood to return to the distal arterial tree after traversing the flow meter the peripheral resistance was left essentially undisturbed and the effects of an arteriovenous fistula (that would have been created had the arterial blood been returned to the iliac vein) were avoided During the determination of the blood flow through the experimental preparation the systemic blood pressure as well as the pulse rate were continuously recorded through a carotid cannula Measures were taken when needed to keep the hemodynamic conditions during each experiment as constant as practicable During arterial occlusion necessary for the construction of the various anastomoses the distal arterial beds were heparinized and systemic heparinization was practiced during the actual meter runs (by the intravenous administration of 2 mg of heparin/kg of body weight) The glass surfaces of the instruments in contact with blood had been coated with silicone In making the vascular anastomoses the conventional clinical instruments and techniques were employed

For each experiment 2 to 10 animal preparations were made Every recorded result was the calculated mean value of at least 6 paired determinations on the same preparation If the serial readings in a given experiment deviated by more than 15% the experiment was discarded As a further control at the completion of each experiment blood flow was measured by a 15 second collection of blood from the distal ends of the iliac trunks Thereafter the anastomoses were taken down and inspected for the presence of stenosis or

thrombotic occlusion The presence of such artefacts invalidated the experiment

The values of the blood flow on the experimental side were expressed as percentages of the flow determined simultaneously on the control side

DISCUSSION

Description of groups of experiments. The subsidiary problems in each of the four groups of experiments mentioned above were as follows *Group 1. Type of anastomosis* (a) Comparison of end to-end anastomosis to intact control. (b) Comparison of end to-side anastomosis (end of graft to side of host) to end to-end anastomosis of the control with variations in the angle of anastomosis on the experimental side. (c) Comparison of side to-end anastomosis (side of graft to end of the host) to end to-end anastomosis of the control with variations in the angle of anastomosis on the experimental side.

Group 2. Bypass grafting. (a) Comparison of bypass graft to intact control. (b) Comparison of bypass graft to direct replacement of control (with end to-end anastomosis both proximally and distally) (c) Comparison of bypass graft inserted with a special anastomotic opening to direct replacement of control

Group 3. The effect of variation in graft diameter). (a) The effect of an increase in diameter of 60% over the control. (b) The effect of an increase in diameter of 100% over the control (c) The effect of an increase in diameter of 140% over the control (d) The effect of an increase in diameter of 170% over the control —In all these experiments the experimental implant was a homograft of human origin and the anastomoses were end to-side.

Group 4. The effect of luminal surface characteristics of prosthesis (a) Comparison between elastic dacron prosthesis and human femoral arterial graft (b) Comparison between crimped braided nylon prosthesis (Edwards-Tapp) and human femoral arterial graft.

RESULTS AND COMMENTS

The findings of these experiments are listed in summary form in Tables 1 to 4

The results of subgroup (a) of the experiments under *Group 1* undertaken to explore the effects of the type of anastomosis on volume flow of blood showed that a properly performed end to-end anastomosis did not appreciably interfere with blood flow. The remaining experiments in this group aimed at gathering information about the possible influence of two technical details concerned with the performance of end to-side anastomoses the angle at which the arteries forming the anastomosis were approximated and the size and shape of the anastomotic opening in the recipient artery. One fact stood out rather conspicuously, namely, that the more acute (or smaller) the angle between the component arteries, the more efficient was the anastomosis A longer anastomotic opening in the recipient vessel likewise increased the efficiency of the anastomosis as did also the removal of a narrow brim of the edge of the opening in the wall of the recipient artery resulting in a widening of the slit It should be noted, however, that as regards the length of the anastomotic opening in end to-side anastomoses, it is closely dependent on the angle of union of the component arteries, that is to say, the longer

opening will require a sharper angle on the slant of the cut end the graft which, in turn, results in a narrower angle of union. With respect to the increased efficiency of the wider anastomoses obtained by excising a rim of the recipient arterial wall, it should be pointed out that this technical step is not

Table 1. Group 1. Effect of Type of Anastomosis on Blood Flow

SUBGROUP	TYPE OF ANASTOMOSIS CONTROL SIDE	EXPER. SIDE	NO. OF EXP.'S	BLOOD FLOW ON CONTROL SIDE ml./min. RANGE	MEAN	BLOOD FLOW ON EXPERIMENTAL SIDE ml./min. RANGE	MEAN	FLOW ON EXPER. SIDE EXPRESSED AS % OF FLOW ON CONTROL SIDE RANGE	MEAN
(a)	Intact Vessel	End to end	2	28.7 to 53.6	37.4	28.0 to 56.2	36.7	96 to 99	97
(b)	End to end	End to side with varying angles	7	17.3 to 73.8	38.8	16.8 to 79.8	33.1	60 to 100	88
(c)	End to end	Side-to-end with varying angles	3	43.2 to 78.9	54.5	37.5 to 46.1	41.9	55 to 98	81

Note: End to side denotes an anastomosis of the end of the graft to the side of the host artery; side to end, the opposite.

Table 2. Group 2. Blood Flow Through Bypass Grafts

SUBGROUP	OPERATIVE TECHNIQUE CONTROL	IMPLANT	NO. OF EXPER.	BLOOD FLOW ON CONTROL SIDE ml./min. RANGE	MEAN	BLOOD FLOW ON EXPER. SIDE ml./min. RANGE	MEAN	FLOW IN IMPLANT EXPRESSED AS % OF FLOW IN CONTROL RANGE	MEAN
(a)	Open Vessel	Bypass	3	32.7 to 51.4	43.1	22.2 to 40.8	27.0	50 to 79	63
(b)	Direct Segmntl Replacement	Bypass	4	19.8 to 72.0	44.3	13.4 to 55.2	36.5	63 to 94	79
(c)	Direct Segmntl Replacement	Bypass Special Anastomoses	3	25.9 to 57.0	42.2	30.0 to 75.4	52.0	106 to 140	121

Table 3. Group 3. Effect of Variation in Graft Diameter on Blood Volume Flow

SUBGROUP	CONTROL TO GRAFT RATIO	NO. OF EXPER'S	VOLUME FLOW IN CONTROL ml./min. RANGE	MEAN	VOLUME FLOW IN GRAFT ml./min. RANGE	MEAN	VOLUME FLOW IN GRAFT AS % OF FLOW IN CONTROL RANGE	MEAN
(a)	1:1.6	10	12.2 to 24.9	18.4	13.8 to 31.6	22.3	112 to 139	120
(b)	1:2.0	10	13.2 to 24.7	23.4	21.2 to 41.6	32.7	149 to 187	167
(c)	1:2.4	6	17.0 to 52.1	25.5	14.9 to 48.4	23.2	81 to 104	90
(d)	1:2.7	4	18.3 to 27.3	22.0	14.5 to 20.4	16.4	72 to 79	76

Note: The grafts studied were fresh human femoral arteries. The length of the grafts was uniform (5 cm.). Both proximal and distal anastomoses were end to side.

Table 4 Group 4 Effect of Luminal Surface Characteristics of Prostheses on Blood Volume Flow

SUBGROUP	TYPE OF PROSTHESIS	NO OF EXPS	VOLUME FLOW IN CONTROL *ml/min* RANGE	MEAN	VOLUME FLOW IN PROSTHESIS *ml/min* RANGE	MEAN	VOLUME FLOW IN PROSTHESIS AS % OF FLOW IN CONTROL RANGE	MEAN
(a)	*Elastic Woven* Dacron	*10*	*2?9 to 59.7*	*31.1*	*21.6 to 51.3*	*30.3*	*81 to 96*	90
(b)	Crimped Braided Nylon (Edwards Tapp)	10	22.0 to 40.8	31.5	16.1 to 30.0	25.0	73 to 89	80

Note The controls were human femoral arterial grafts All anastomoses were end to side Length of all implants 5 cm diameter of all implants 8 mm

usually feasible in clinical practice where one almost always deals with arteries so diseased as to preclude this maneuver The unfavorable effect of the angulation of the arteries forming an anastomosis found in these studies is in agreement with the general principles of fluid mechanics [4] According to these principles at the point of angular union of two pipes the fluid flow becomes turbulent and a loss of pressure head takes place which is more marked the greater is the angle of the union

In *Group 2* experiments for the study of the efficiency of bypass grafting *in the first two of these subgroups* (a and b) the bypasses were constructed in an identical manner In the preparations the variables were on the control side which in subgroup (a) was left intact and in subgroup (b) had a segmental replacement with proximal and distal end-to-end anastomoses It should be made clear that the segment of artery bypassed by the graft was in all cases of Group 2 *completely occluded* The *volume flow through the bypass* was reduced in subgroup (a) to 63% and subgroup (b) to 79%) This reduction can be readily explained by the phenomena known as entrance and exit losses of fluid flow [4] The findings in subgroup (c) were of interest in these experiments the anastomoses between the grafts and the host artery were performed at a very acute (small) angle and with the *additional* maneuver of excising a rim of the opening in the recipient artery, and significant increase (+21%) in flow was observed in the bypass

The experiments in *Group 3* dealing with the effect of the variation of graft diameter demonstrated that an increase in diameter up to twice the diameter of the control brings about an augmentation of volume flow which however is not a linear change With an increase of 60% in the diameter an average increase in flow of 20% was found while an increase in diameter of 100% produced an increase in flow of 67% *Increasing* the diameter by a factor of 2.1 and 2.7 resulted in a decrease of volume flow This decrease was undoubtedly caused by the entrance and exit losses brought about by the *turbulence in flow that supervenes* when the inequality between the diameters of the graft and the host arteries exceeds a critical value From the point of view of the practical application of these findings it must be further *borne in mind that* any increase in the diameter of a graft in comparison to the diameter of the host artery results in some loss of velocity of flow and this may be of greater importance than the gain in volume flow since it

orifice size an increase in the aorticoventricular in increase in regurgitant flow.

5 Bradycardia with resulting prolongation of diastole causes an increased regurgitant flow per beat even in the presence of a lowered aorticoventricular pressure gradient

SUMMARY

Thoracic aortic blood flow pulses in dogs with acute and chronic aortic valve incompetence were measured with an electromagnetic flow meter. Central aortic pressures, left ventricular pressures, and left ventricular myograms were recorded simultaneously. With small to moderate degrees of incompetence, retrograde aortic flow persisted through diastole. The prime factors determining the magnitude of regurgitant flow were the regurgitant orifice size, the aorticoventricular diastolic pressure gradient and the length of diastole Compensation for the lesion appeared to be achieved by an increase in the duration of ventricular ejection time and an increase in heart rate

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SURGICAL TREATMENT OF AORTIC INSUFFICIENCY BY OPEN PLASTIC REVISION OF THE TRICUSPID AORTIC VALVE TO A BICUSPID VALVE *

JOSEPH J GARAMELLA, JAMES G ANDERSEN, RUBEN OROPEZA, ANGELO VELOSO, RANGASWAMY NAIDU, AND LYLE J. HAY

Available methods of controlling aortic insufficiency with prosthetic valves[1] have the limitations of 1) partial control of the regurgitation because of the distal location of the prosthesis, 2) foreign body thrombosis, 3) aneurysm formation, 4) infection, 5) the traumatic cellular effect of an artificial valve in long continued use The technical problems related to placement of supra or subcoronary prostheses have not been surmounted to satisfaction Likewise, circumferential pursing techniques attempting to reduce aortic valve circumference, pericardial pedicles, vein grafts, and homologous aortic valve grafts have been disappointing

* From the Jay Phillips Laboratory, Mount Sinai Hospital, and the Department of Surgery, University of Minnesota Supported by U S Public Health Grant No H 2610(C2) and Cardiac Research Committee and Coronary Disease Research Fund, University of Minnesota, and the Hartford Foundation With the technical assistance of Dr Richard Ernst in the photographic studies with the pulse duplicator

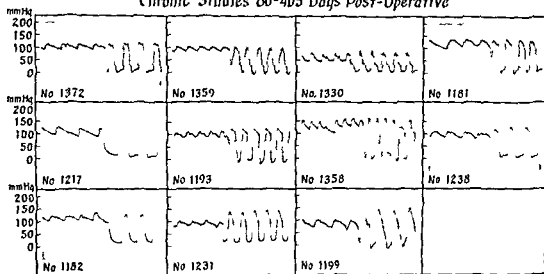


Fig 4

Pathologic Studies. Autopsy specimens with rheumatic aortic insufficiency have been studied Using a hydraulic pulse duplicator it appears that the non coronary cusp interferes with the coaptation of all 3 aortic cusps Resection of the noncoronary cusp with formation of a bicuspid aortic valve provides excellent coaptation of the 2 remaining coronary cusps and appears to minimize aortic regurgitation

Clinical Cases. Two patients have been accepted as candidates for this operation The first patient, a 21 year old woman in severe congestive failure despite medical management and prolonged bed rest, proved to have a congenital bicuspid valve with perforation of one cusp and fissuring of the other due to subacute bacterial endocarditis Suture correction of the defects were unsuccessful and the patient died the evening of surgery

The second patient, a 19 year old man, had aortic insufficiency for many years Two months prior to surgery he suffered acute pulmonary edema There was progressive cardiac enlargement and A V dissociation Prior to surgery combined left and right heart catheterization were performed Symptoms increased The cardiac silhouette increased notably Surgery was elected Exploration disclosed chronic hemopericardium 900 cc of old bloody fluid in the pericardial sac A shaggy pericarditis was present Shortly following pericardiotomy ventricular fibrillation occurred Electrical defibrillation was accomplished Following a period of observation the operation was continued At the time of performing elective potassium asystole cardiac irregularities increased The heart appeared to stop of its own accord as only 25 cc of 2½% potassium citrate produced arrest A bicuspid aortic valve was fashioned and the coaptation of the 2 coronary cusps appeared excellent However following the plastic operation, sustained effective ventricular contractions could not be obtained Autopsy showed a heart weighing 750 gm The reconstructed bicuspid aortic valve appeared effectively competent Severe coronary disease with multiple old infarcts were found

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A METHOD FOR REPLACING THE AORTIC ARCH IN DOGS MAINTAINING NORMAL BLOOD PRESSURES WITH TWO PUMPS AND AVOIDING PROLONGED USE OF AN OXYGENATOR *

FRANK E JOHNSON LOUIS D PHILIPP JOSEPH A DOWLEN
OREST N FILIPOVICH LEWIS E HUGHES AND CLAUDE R HITCHCOCK

The technical factors involved in resection of the aortic arch pose a challenging problem During recent years others have accomplished this operation in dogs utilizing special vascular clamps permitting rapid anastomoses of nondiseased arteries In clinical experience the total aortic arch has been replaced by means of shunts interposed between major vessels or by the prolonged use of a pump oxygenator The diseased human arteries requiring surgery do not lend themselves to rapid anastomosis by special clamps and the technique of end to side bypass shunts is cumbersome frequently technically difficult to accomplish and often impossible to apply Particularly in acute dissecting aneurysms is the foregoing true We believe a method of resection and replacement of the aortic arch permitting careful unhurried end to-end suture anastomoses will be most consistently successful

In attempting to solve this problem we drew upon our laboratory and clinical experience in replacement of the descending thoracic aorta using a method of controlled bypass (sigmamotor pump) from the left atrium to the femoral artery The possibility of extending this method to permit replacement of the aortic arch by adding a cephalic circuit seemed to be worthy of investigation The attractive features of such an approach are 1) avoiding prolonged use of an oxygenator 2) ready application of this procedure under emergency conditions 3) permitting a series of accurate end to end anastomoses to be fashioned without hurry

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* From the Research Laboratories of the Minneapolis General Hospital and the University of Minnesota

METHOD

Obviously of prime concern was the problem of supplying sufficient oxygenated blood to the peripheral circulation while the total arch and its *branches were being replaced Our solution is diagramed in Figure 1* Oxygenated blood from the left atrium is collected in a reservoir and perfused to the body and head of the dog by means of two sigmamotor pumps While this technique provides adequate peripheral circulation for prolonged periods of time a second and more troublesome problem has been the occurrence of acute left ventricular strain failure and cardiac arrhythmia subsequent to cross clamping of the ascending aorta just distal to the ostia of the coronary arteries

We have been able to accurately control aortic root pressures during cross clamping of the descending thoracic aorta by varying the rate of gravity removal of blood from the left atrium however with occlusion of the ascending aorta variations in the rate of gravity flow from the left atrium have not been adequate to prevent wide fluctuations in aortic root pressures and severe left ventricular hypertension has resulted Intraaortic pressures at the root have been measured by means of a catheter threaded to the supravalvular area through the left subclavian artery and pressures ranging from 60 mm Hg to 220 mm Hg have been recorded following interruption of flow in the ascending aorta The possibilities of utilizing this catheter to control such wide fluctuations became evident Figure 2 portrays the stepwise performance of

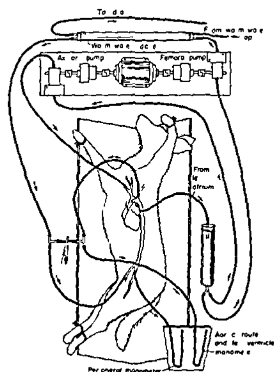


Fig 1 Diagram of circuit used to perfuse total animal during aortic arch replacement The clamp is used during measurements of pressures only

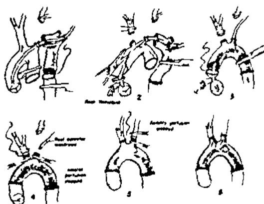


Fig 2 Stepwise removal of total aortic arch utilizing the aortic root catheter to level off wide fluctuations in aortic root pressures Not as successful as procedure diagramed in Figure 3

resection and replacement of the aortic arch with a homograft utilizing the root catheter to "bleed off" the proximal aorta during periods of hypertension or to provide a source of blood for coronary flow during periods of hypotension Even though this technique smoothed out the extreme fluctuations of pressure in the proximal aorta, it did not prevent the appearance of ventricular strain and dilatation during the time involved between steps 2 and 4 in Figure 2 (20 to 24 minutes)

Total Left Ventricular Bypass More recently we have modified the system so that the left atrial catheter passes through the mitral valve and drains the left ventricle as well as the atrium during that period of time when the ascending aorta is occluded with a tape ligature A negative pressure (20 cm H_2O) is applied to this catheter during this portion of the perfusion in an effort to empty these chambers almost completely Blood is then pumped to the coronary arteries through the aortic root catheter obviating the need for the left ventricle to perform work With this method it is possible to cross clamp the aorta 2 cm above the coronary ostia for as long as 30 minutes without apparent deleterious effects upon the heart During this time the heart remains of good color and maintains a regular rhythm with no evidence of distention or failure As the left ventricular bypass is discontinued it appears to be important to restore the flow from the atrium to the ventricle in gradually increasing amounts and thus only slowly return a full work load to the left ventricle

Stepwise Resection of Aortic Arch The current technique used in our laboratory is represented in Figure 3 Drawing 1 indicates perfusion of the head and right upper extremity by means of normal flow through the *ascending* aorta perfusion of the lower half of the body through the femoral catheter, and performance of the distal anastomosis Drawing 1A indicates the time in the procedure when the left atrial catheter is advanced into the left ventricle and total ventricular bypass instituted with perfusion of the coronaries via the root
and 4 *indicate the peri*
through the right axill
the left ventricular catheter back into the left atrium immediately prior to reestablishing flow in a normal manner through the graft to the *coronaries* and lower portions of the body In Drawing 5 the brachiocephalic artery anastomosis has been completed and the left subclavian artery is being *anastomosed* all perfusions having been stopped at completion of the

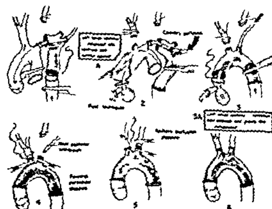


Fig 3 Sequence of surgical steps in removing total aortic arch utilizing aortic root catheter to perfuse coronaries during tape ligature of ascending aorta Note 1A and 3A as periods delineating time of total left ventricular bypass.

brachiocephalic anastomosis Drawing 6 depicts the completed operation and all perfusion catheters have been withdrawn from the animal The technique of supporting the various major anastomoses with a small band of Ivalon or dacron has minimized blood loss and decreased perfusion time and operating time

RESULTS

Prior to utilization of the root catheter technique for controlling pressure within the root of the aorta during occlusion of the ascending aorta we were able to complete only one arch replacement of 14 attempted Since the aortic root catheter technique has been used 26 arch replacements have been completed in 39 attempts (67%) The addition of the total left ventricular bypass for the short period of time (20 minutes) represented in Drawings 2 and 3 *seems to provide significant advantages in that the left ventricle is put under* less stress and as a result appears to be in better condition to resume its function at the completion of the operation Final results with this apparent refinement in technique are not available at the time of writing

DISCUSSION

The experience in our laboratories with total resection of the aortic arch in dogs has been presented From our studies it appears important to control pressures within the aortic root and left ventricle during cross clamping of the ascending aorta and a technique of catheter control to the root of the aorta has been presented The most satisfactory results have been obtained with a technique utilizing total left ventricular bypass during that portion of the operation when the ascending aorta is occluded with a tape ligature just distal to the ostia of the coronary arteries

The application of this surgical procedure in humans should not be difficult since the insertion of a second cephalic perfusion catheter into the left external carotid artery should suffice along with a catheter in the right axillary artery to totally perfuse the head during the periods of the surgery when the flow through the carotids is interrupted We believe that a system utilizing two pumps is beneficial since differential rates of flow can be provided to the head and the remainder of the body in this manner

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EXPERIMENTAL EVALUATION OF THE TOXICITY OF HYPAQUE, UROKON AND THOROTRAST INCIDENT TO THEIR USE AS AORTOGRAPHIC CONTRAST MEDIA*

DUNCAN A. KILLEN, EDWARD M. LANCE AND GUY OWENS

The majority of fatal and severe nonfatal complications of abdominal aortography result from the toxic effects of the contrast medium on the kidneys and spinal cord. In recent years urokon 70% (sodium acetrizoate) has been the organic iodide compound of choice for use in aortography.[1] This study compares the neurotoxic and nephrotoxic potential of urokon 70% with that of hypaque M 90% (sodium and N methylglucamine diatrizoate) and thorotrast 25% (thorium dioxide), two contrast media of reportedly low acute tissue toxicity.[2, 3]

METHOD

The test animals were unselected mongrel dogs. Two standardized methods of injection of contrast medium were used. Features common to both methods were the use of nembutal anesthesia (30 mg./kg.) and the rapid manual injection of the contrast medium under direct vision at laparotomy with the animal in the supine position.

One group of animals received aortic injections performed as follows: the tip of an 18 gauge needle was slanted cephalad after puncture of the anterior wall of the aorta 1 cm. below the origin of the left renal artery, and 15 cc. of contrast medium were injected over a 5 second period.

In a second group of animals injections were directed into a pair of lumbar arteries in the following manner: the first pair of lumbar arteries caudad to the origin of the left renal artery and the corresponding aortic segment were temporarily isolated from the circulation by appropriate application of Potts ductus clamps. Amounts of contrast medium varying from 2 cc. to 30 cc. were injected into the aortic segment. Release of the proximal ductus clamp was effected before release of the distal clamp to insure complete lumbar artery dissemination of the injected material.

Hind limb neurological deficit was assessed in all the animals following recovery from anesthesia.

In the animals receiving aortic injections, renal injury was evaluated clinically by means of serial nonprotein nitrogen determinations.

Pertinent microscopic examinations of the spinal cords and kidneys augmented the clinical studies.

RESULTS

In the aortic injection group paraplegia occurred consistently following instillation of urokon, while no neurological deficit resulted from the injection of hypaque or thorotrast (Fig. 1).

Lumbar artery injections of as little as 2 cc. of urokon caused paraplegia, but similar injections of as much as 30 cc. of hypaque or thorotrast caused no detectable motor deficit (Fig. 2).

* From the S. R. Light Laboratory for Surgical Research and the Departments of Surgery and Anatomy, Vanderbilt University School of Medicine, Nashville. Supported in part by the Research and Development Division, Office of The Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-697.

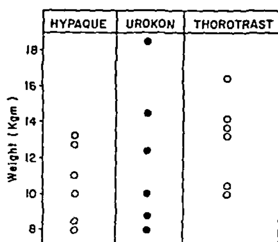


Fig 1 Neurological damage resulting from intraaortic injection of 15 cc of contrast media Note lack of influence of animal weight

AMOUNT (cc)	HYPAQUE	UROKON	THOROTRAST
30	O O O	——	O O O
15	O	●	O
5	——	● ●	——
2	——	O ● O	——

● Paraplegia
O No Neurological Deficit

Fig 2 Neurological damage resulting from segmental arterial injection of varying amounts of contrast media

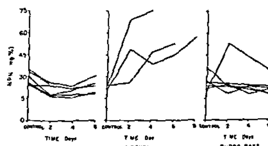


Fig 3 Serial nonprotein nitrogen values following intraaortic injection of hypaque-M 90% urokon 70% or thorotrast 25%

Urokon paraplegia was usually heralded by an immediate extensor rigidity and myoclonic seizure of the hind limbs A concomitant observation was a potentiation of the barbiturate anesthesia by urokon

The paraplegia itself was characterized by flaccidity of the hind limbs loss of sphincter control and in some instances priapism Clinical appraisal of the paraplegia as late as 5 weeks postinjection failed to reveal any neurological improvement

Microscopic examination of spinal cords from the paraplegic animals revealed massive necrosis and dissolution of the central gray matter

Serial nonprotein nitrogen values of the animals surviving aortic injections are recorded in Figure 3 The NPN values of those animals receiving urokon uniformly rose to abnormally high levels The rapid deterioration and death of the animals receiving aortic injections of urokon were attributed to acute renal damage (only 1 of the 6 animals survived more than 7 days) A transient rise of the NPN value in 1 animal which had received thorotrast constituted the only evidence of possible renal injury resulting from the insult by either hypaque or thorotrast

Pathologically the lesions of urokon nephropathy was that of acute tubular necrosis

DISCUSSION

There would seem to be adequate reason to temper enthusiasm for the use of urokon as an aortographic contrast medium in view of its observed

cytotoxic effects, especially with regard to the central nervous system and kidney.

Although there is no immediate reaction to thorotrast 25%, the late complications incident to its use, namely extravasation granuloma, aplastic anemia, and sarcomatous degeneration of the liver, are documented.[4] These late complications prohibit its general use as an aortographic contrast medium. It seems safe for use in elderly patients in those situations permitting surgical exposure of the aorta. Its radiopacity is not as great as that of urokon 70%.

A consideration of this study indicates that hypaque-M 90% exhibits far less direct affinity for cells of the central nervous system and kidney than does urokon 70%. This is surprising in view of the fact that both compounds are derivatives of triiodobenzoic acid and are structurally closely related. The iodine contents of these mediae are essentially the same, and therefore, the radiographic characteristics are identical. The main deterrent to widespread clinical use of hypaque-M 90% is the great viscosity which prevents rapid injection. This disadvantage may be partially vitiated by the use of high pressure injection systems of low resistance or dilution of the 90% solution. These maneuvers have aided in securing satisfactory aortograms experimentally but final evaluation must await increased clinical trial.

CONCLUSIONS

Urokon 70%, as opposed to hypaque-M 90% and thorotrast 25%, exhibits an immediate and potent toxic effect on the kidney and central nervous system.

Thorotrast 25% is not generally acceptable for aortography because of its delayed toxic manifestations and inferior radiopacity.

Hypaque-M 90% gives good radiocontrast, has minimal tissue toxicity, but its marked viscosity hinders rapid injection.

Mallinckrodt Chemical Works (Urokon), and Winthrop Laboratories (Hypaque-M) generously furnished supplies of their products.

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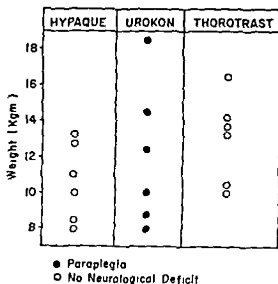


Fig 1 Neurological damage resulting from intraaortic injection of 15 cc of contrast media Note lack of influence of animal weight

AMOUNT (cc)	HYPAQUE	UROKON	THOROTRAST
30	○ ○ ○	—	○ ○ ○
15	○	●	○
5	—	● ●	—
2	—	○ ● ○	—

● Paraplegia
○ No Neurological Deficit

Fig 2 Neurological damage resulting from segmental arterial injection of varying amounts of contrast media

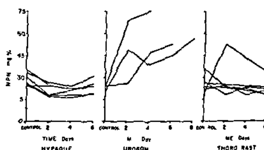


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There would seem to be adequate reason to temper enthusiasm for the use of urokon as an aortographic contrast medium in view of its observed

cytotoxic effects, especially with regard to the central nervous system and kidney.

Although there is no immediate reaction to thorotrast 25%, the late complications incident to its use, namely extravasation granuloma, aplastic anemia, and sarcomatous degeneration of the liver, are documented.[4] These late complications prohibit its general use as an aortographic contrast medium. It seems safe for use in elderly patients in those situations permitting surgical exposure of the aorta. Its radiopacity is not as great as that of urokon 70%.

A consideration of this study indicates that hypaque-M 90% exhibits far less direct affinity for cells of the central nervous system and kidney than does urokon 70%. This is surprising in view of the fact that both compounds are derivatives of triiodobenzoic acid and are structurally closely related. The iodine contents of these mediae are essentially the same, and therefore, the radiographic characteristics are identical. The main deterrent to widespread clinical use of hypaque-M 90% is the great viscosity which prevents rapid injection. This disadvantage may be partially vitiated by the use of high pressure injection systems of low resistance or dilution of the 90% solution. These maneuvers have aided in securing satisfactory aortograms experimentally but final evaluation must await increased clinical trial.

CONCLUSIONS

Urokon 70%, as opposed to hypaque-M 90% and thorotrast 25%, exhibits an immediate and potent toxic effect on the kidney and central nervous system.

Thorotrast 25% is not generally acceptable for aortography because of its delayed toxic manifestations and inferior radiopacity.

Hypaque-M 90% gives good radiocontrast, has minimal tissue toxicity, but its marked viscosity hinders rapid injection.

Mallinckrodt Chemical Works (Urokon), and Winthrop Laboratories (Hypaque M) generously furnished supplies of their products

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THE INFLUENCE OF LIGATION OF INTERCOSTAL ARTERIES ON PARAPLEGIA IN DOGS*

FRANK C. SPENCER AND JACK M. ZIMMERMAN

A serious complication following excision of a thoracic aneurysm is paraplegia. This complication, which is a result of spinal cord ischemia, may result from two causes. Ischemic injury can result from the decreased blood flow during the period of aortic occlusion required for excision of the aneurysm or it may result from ligation of intercostal arteries arising from the aneurysm. The recently developed technique of pumping blood from the proximal to the distal aorta during the period of aortic occlusion has significantly decreased the frequency of paraplegia.[1] When paraplegia occurs despite the use of a bypass technique, it probably is a result of excision of intercostal arteries and theoretically can be prevented only by preservation of the intercostal arteries. The present study was designed to evaluate the importance of intercostal arteries in the blood supply of the spinal cord of the dog and to evaluate techniques for preserving intercostal arteries during excision of thoracic aneurysm.

METHOD

Twenty seven mongrel dogs, weighing 10 to 15 kg., were operated upon under endotracheal ether anesthesia. The intercostal arteries were exposed through incisions in the sixth and tenth left intercostal spaces. As the first two or three intercostal arteries usually did not originate from the aorta, the left subclavian artery was routinely ligated in order to remove the highest left thoracic arterial supply. The intercostal artery to the fourth thoracic interspace was usually the first one originating from the aorta. Distally, the ligations were carried through the first lumbar artery; this artery was usually smaller than the intercostal arteries. Additional lumbar arteries were ligated in the first 2 animals operated upon before it was found that such extensive ligations were not necessary to produce paraplegia.

After the individual arteries were divided, the thoracic aorta was completely mobilized to be certain that all intercostals were divided. The aorta was never occluded, however, so that any neurologic injury that developed could result only from division of the intercostal arteries. Following closure of the chest incision, the animals were observed for signs of neurological injury.

In 4 animals the thoracic aorta was excised and replaced with an aortic homograft while a shunt of plastic tubing between the subclavian or carotid artery and the femoral artery provided blood to the distal aorta. An elliptical segment of aortic wall containing two pairs of intercostal arteries was preserved and anastomosed to an oval opening made into the side of the homograft. All of the other intercostal arteries, the first lumbar arteries, and the left subclavian artery were divided. When an ellipse of aortic wall from which the intercostal arteries arose was used, the technical difficulty of directly anastomosing a small intercostal artery was avoided.

* From the Department of Surgery, Johns Hopkins University School of Medicine, Baltimore. Aided by Grant H 226 from the National Heart Institute, U. S. Public Health Service.

RESULTS

The results obtained are shown in Table 1. In the control group of 7 dogs all intercostal arteries, the left subclavian artery, and the first lumbar artery were divided; in 2 of these dogs additional lumbar arteries were divided. Complete paralysis of the hind legs was present in all of the animals on recovery from anesthesia. The animals had normal use of the forelegs but most of them died within 18 hours, apparently from the extensive neurological injury.

In 12 dogs ligations similar to those in the control group were done except that two pairs of intercostal arteries were left intact at various levels. As shown in Table 1, the tenth and eleventh intercostal arteries were preserved in 7 animals, the eleventh and twelfth intercostals in 2, the seventh and eighth in 2, and the twelfth and thirteenth in one. One of the 12 animals had some weakness in 1 leg; none of the other 11 showed any neurological injury.

Table 1. The Influence of Intercostal Artery Ligation on Paraplegia†

INTERCOSTAL ARTERIES PRESERVED	NO. OF DOGS OPERATED UPON	NO. OF DOGS WITH PARAPLEGIA
None	7	7
T 12 and 13	1	0
T 11 and 12	2	0
T 10 and 11	7	0
T 7 and 8	2	0
T 1 through T 9	2	0 ††
None	2	1 ††

† The left subclavian artery and the first lumbar arteries were also ligated in all dogs except as indicated.
†† Left subclavian not ligated.

In 2 animals ligation of the arteries from the tenth thoracic through the first lumbar did not cause any injury. This was done to be certain that the intercostal arteries below the ninth intercostal are not of critical importance to the circulation of the spinal cord. In 2 animals the importance of ligation of the left subclavian artery in causing paraplegia was tested by ligating all of the intercostal arteries but not the subclavian artery. One of the 2 animals was paraplegic, the other was not.

Several technical difficulties were encountered in the 4 operations in which the thoracic aorta was replaced with a homograft and a segment of aortic wall from which intercostal arteries arose anastomosed to the side of the homograft. The first 3 operations were terminated by extensive bleeding from the suture lines, perhaps from inadequate neutralization with protamine of the heparin given when the subclavian femoral shunt was established. Following several modifications of the operative technique, the last animal operated upon survived without signs of neurological injury.

DISCUSSION

The results show that extensive ligation of the intercostal arteries may [illegible]

reported by Lam[2] in 1952 who ligated intercostal arteries in 10 dogs with paraplegia resulting in 2 The exact extent of the ligations was not described No other report concerning the influence of intercostal arteries on paraplegia in dogs was found in the surgical literature

The applicability of these experimental findings to man is limited by two considerations Although the intercostal artery which was preserved was not of critical importance in the dog a similar situation is probably not true in man because only about one fourth of the intercostal arteries have radicular branches that supply the spinal cord[3] If intercostal arteries were preserved in man it would not be possible to be certain that the arteries preserved were those with radicular branches supplying the spinal cord Also a few successful resections of the entire descending thoracic aorta have been recently done with bypass techniques[1,4] It cannot be determined whether the absence of neurological injury in these extensive resections was due to a fortunate location of critical radicular arteries that were not excised or whether the gradual *thrombosis of radicular arteries arising from the aneurysm had permitted the* development of collateral circulation from radicular arteries arising from adjacent areas of the aorta

The findings in dogs however that preserving only two pairs of intercostal arteries is sufficient to prevent paraplegia emphasizes the importance of preserving intercostal arteries when possible If the arteries can be preserved only by an anastomosis to the aortic graft the technique described in these experiments may be applicable

SUMMARY

Ligation of all of the intercostal arteries as well as the left subclavian artery and the first lumbar arteries resulted in paraplegia in 7 dogs Paraplegia was avoided in 12 dogs similarly operated upon by preserving two pairs of intercostal arteries The level at which the intercostal arteries were preserved was not critical A technique for preservation of intercostal arteries during excision of the thoracic aorta was evaluated in 4 dogs A small segment of aortic wall from which the intercostal arteries arose was anastomosed to an oval opening made into the side of the aortic homograft

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FURTHER STUDIES OF RENAL AND ADRENAL FACTORS IN THE HYPERTENSION OF COARCTATION OF THE AORTA*

H. William Scott, Jr., Lawrence S. McGee, Jr.,
Robert W. Youngblood, Duncan A. Killen, A. Page Harris
and Edward M. Lance

Previous studies[1-3] have suggested that the kidneys are intimately concerned with both the production and maintenance of the hypertension of coarctation. Experimental constriction of the thoracic aorta in the range of 60 to 80% of its diameter was found to result in a progressive elevation of the carotid arterial pressure to a hypertensive level after an initial depression; femoral arterial pressure was also found to increase. On the contrary, complete occlusion of the aorta immediately below the renal arteries caused no elevation in carotid pressure. In animals with the hypertension of experimental coarctation, transplantation of one kidney to a site above the aortic constriction and contralateral nephrectomy resulted in disappearance of hypertension. The hypertensive pattern following production of experimental coarctation, however, was not altered by transplantation of one kidney to a site above the level of coarctation as long as the other kidney remained below the aortic constriction. When the kidney inferior to the aortic stricture was excised in such experiments there was prompt reversion of the carotid blood pressure to its preoperative control level. These data support the concept that the mechanical stricture of the aorta in coarctation causes hypertension by producing a disturbance in renal hemodynamics similar to that of the Goldblatt kidney.

The present study is an attempt to assess other methods of altering aorticorenal arterial relationships, to examine their effects on blood pressure, and to determine whether or not the adrenals have a role in either the production or maintenance of the hypertension of coarctation by: 1) shifting the origin of the renal arterial blood supply from aorta to iliac artery by means of a long graft; 2) constriction or transection of the aorta between the right and left renal arteries and subsequent removal of the upper kidney (inter-renal coarctation); 3) determination of levels of catechol amines in animals with experimental coarctation and in patients with clinical coarctation before and after operation; 4) assessment of the effect of bilateral total adrenalectomy on the hypertension of experimental coarctation.

METHOD

In the animal experiments healthy adult mongrel dogs were used. Prior to any experimental aorticorenal arterial alteration one of the carotid arteries was transplanted subcutaneously to facilitate subsequent needle puncture. Blood pressure was determined in the unanesthetized animal by direct puncture of the femoral and carotid arteries using a 20 gauge needle attached

* From the S. R. Light Laboratory for Surgical Research, Department of Surgery, Van[illegible] ... by a research grant No. H 2366 ... With the assistance of Donald [illegible] ... the Vanderbilt University Hos[illegible] ... [illegible] performed the catechol amine determinations.

to a mercury manometer A sufficient number of blood pressures were taken to insure their constancy before any operative procedure was undertaken Intravenous sodium pentobarbital anesthesia and aseptic surgical technique were used in all operative procedures Postoperative observations were made in chronic survivors over periods of 2 to 15 months

RESULTS

In general, most of the animals which survived the operative procedure and the immediate postoperative period remained in good condition A total of 72 animals were used in the experiments, of which 22 were long range survivors

Animals with Origin of Renal Arterial Blood Supply Shifted from Aorta to Iliac Artery by Means of a Graft. The purpose of this experiment was to determine if, by changing the angle between renal arterial and aortic axial blood flows, the Bernoulli hydrodynamic principle might operate in the genesis of hypertension of renal origin In 11 dogs the left renal artery was ligated and divided A freeze dried canine carotid homograft was interpolated between the distal end of the left renal artery and the side of the left common iliac artery, thus creating an extremely acute angle between axial blood flows After 5 to 7 days the right kidney was removed Nine animals died in the early postoperative period of thrombosis of the graft In the 2 long range survivors arterial pressures were measured at frequent intervals for 15 months and hypertension did not develop in either animal Lumbar aortograms prior to sacrifice in each instance revealed excellent visualization of the patent grafts with no constriction or evidence of impaired renal arterial blood flow Histologic examination at sacrifice revealed no alterations in renal parenchymal structure

Animals with Aorta Transected or Constricted between Right and Left Renal Arteries and Subsequent Removal of Upper Kidney (Inter Renal Coarctation). The purpose of this experiment was to determine whether severe constriction or transection of the abdominal aorta between the renal arteries and subsequent removal of the superior kidney would result in hypertension Thirty nine animals were used and 10 successful preparations were obtained for study In preparing 7 of these animals Potts ductus clamps were applied obliquely across the aorta between the right and left renal arteries the aorta was divided and each end closed with arterial silk using care to avoid any encroachment on the origin of either renal artery *In 3 other animals* 70% *to* 80% constriction of the aorta at a site between the origin of the right and left renal arteries was accomplished by partial division and suture closure of the incised aortic wall *In these 10 animals the kidney above the site of transection* or constriction was removed 13 to 76 days later, leaving the remaining kidney *in situ* immediately below the point of aortic occlusion The typical *changes in carotid and femoral pressures* which followed these surgical alterations are seen in Figure 1 Weakness of hind legs was commonly encountered immediately after operation but gradually abated and there was no permanent paraplegia in the survivors Seven of 10 dogs developed sustained hypertension of the coarctation type In general, the arterial pressure became elevated prior to removal of the upper kidney but a more consistent hypertensive plateau was achieved after upper nephrectomy In 3 dogs with the typical sustained hypertensive response to this experiment a reversion to

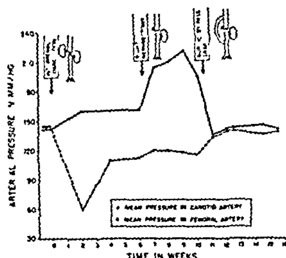


Fig 1 The characteristic changes that occur in carotid and femoral mean blood pressures in an animal subjected to creation of an "inter renal coarctation", proximal nephrectomy and subsequent correction of the coarctation with a bypass arterial graft

normotension was produced by insertion of a bypass graft between the proximal and distal segments of the divided aorta Three other dogs with interrenal coarctation developed the characteristic differences between carotid and femoral pressures seen in clinical coarctation but little or no consistent elevation of carotid pressure developed during many months of observation

Catechol Amine Levels in Experimental and Clinical Coarctation. Plasma levels of catechol amines were measured by a modification of the fluorimetric method[4] at intervals before and after the production and/or correction of the hypertension of experimental coarctation Plasma levels in 13 dogs with normal arterial pressures ranged from 0 μg/L to 1 1 μg/L and averaged 2 1 μg/L

After development of sustained hypertension in 7 of these dogs plasma levels of catechol amines ranged from less than 1 μg/L to 5 0 μg/L, and averaged 1 9 μg/L After restoration of normotension by aortic bypass graft in 3 of these dogs serum catechol amines averaged 2 4 μg/L

In 5 patients with coarctation of the aorta 24 hour urinary excretion of catechol amines ranged from 22 μg to 64 μg, averaging 39 μg † prior to operation Five to 8 days after operative repair, when arterial pressures were in the normal range in each instance, urinary catechol amines ranged from 30 μg to 85 μg averaging 63 μg

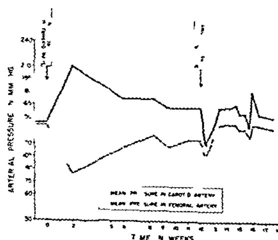


Fig 2 Blood pressure values from an animal subjected to creation of a supradiaphragmatic coarctation followed by bilateral adrenalectomy

† Normal human values for urinary catechol amine output range as high as 150 μg/24 hours by the method used in this study

Effects of Bilateral Total Adrenalectomy on the Hypertension of Experimental Coarctation *In 5 dogs experimental* coarctation was produced by constriction of the aorta above the diaphragm At intervals of 1 to 3 months later when sustained hypertension had become manifest bilateral total adrenalectomy was carried out in each instance Each animal received 100 mg of Solu Cortef and 150 mg of hydrocortisone acetate at the time of operation and thereafter was maintained on stock diet and cortisone acetate 25 mg daily Typical changes in arterial pressures in these animals are seen in Figure 2 A transitory drop in pressure of 4 to 7 days duration occurred following adrenalectomy in all dogs After this interval the hypertensive pattern of coarctation was resumed and maintained

SUMMARY AND CONCLUSIONS

1 Experimental production of an extremely acute angle between aortic and renal arterial axial blood flows failed to produce hypertension

2 Experimental transection or constriction of the aorta between the renal arteries and subsequent removal of the upper kidney resulted in sustained hypertension of the coarctation type in 7 of 10 animals Lack of development of sustained hypertension in 3 animals with this preparation may be attributable to collateral circulation

3 Measurements of plasma and urinary catechol amines in animals and patients with coarctation before and after production or correction of hypertension were all within the range of normal

4 Total bilateral adrenalectomy caused only transitory postoperative reduction *in the hypertension of experimental coarctation*

5 These observations add further evidence in favor of the existence of a renal factor in the hypertension of coarctation and present no evidence suggesting the presence of an adrenal factor

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THE EFFECT ON THE KIDNEYS OF CROSS CLAMPING THE AORTA DISTAL TO THE RENAL ARTERIES *

ERIC MUSARD NANSON, JOHN GRAHAM NOBLE AND HARRY EMSON

Powers, Boba and Stein [1] reported in 1957 that clamping the abdominal aorta of the dog produced distal tubular necrosis of the kidneys They also reported on the effects of renal denervation and of ganglionic blockade in association with aortic clamping The experiments which are here reported were intended to investigate first the production of renal damage by clamping the abdominal aorta and second to test various means of protecting the kidneys from this damage

Unselected healthy mongrel dogs of either sex were used Their body weights averaged 8 kg Anesthesia was obtained by intravenous pentobarbital throughout the experiments A midline abdominal incision was used, and a Potts' type clamp used to occlude the aorta just distal to the origin of the renal arteries A renal biopsy was taken routinely from one kidney and any dogs which showed evidence of renal disease in this biopsy specimen were excluded from the experiment

It was found initially that 4 hours of aortic cross clamping regularly produced distal tubular damage Comparable degrees of damage were produced by 2 hours of clamping, so the shorter period was used in all subsequent experiments

By sacrificing dogs at intervals of 1 to 7 days after operation it was determined that degenerative changes were best seen histologically at the seventh postoperative day Subsequent experiments, therefore, utilized 2 hours of aortic clamping and sacrificing on the seventh post operative day

A control series of 5 dogs had exposure of the abdominal aorta and renal biopsy only The aorta was dissected free as if for clamping but not clamped There was no histological evidence of renal tubular damage in this control series of dogs

The experiments so far confirmed that occlusion of the abdominal aorta produced renal tubular damage

Three possible mechanisms for this damage were considered 1) that the mechanism of the damage may be similar to that occurring in the crush syndrome, 2) that changes in blood pressure in the renal arteries during clamping of the aorta might cause renal tubular damage by a "water hammer" effect, 3) that atheromatous plaques from the aorta proximal to the renal vessels might be dislodged during the period of clamping and cause embolic damage and ischemia in the kidneys

The hind limbs of the dog are ischemic while the aorta is clamped but there is no crushing trauma to large muscle masses in the experiment No histological evidence to implicate either the crush syndrome or arterial embolism was found, and direct measurements of blood pressure in the renal arteries during aortic clamping showed no deviation from control values

It was therefore thought that the renal damage must be due to ischemia caused by arteriolar constriction of reflex origin

It was also found, in further control experiments, that local contusion of

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the aorta, without clamping the vessel, did not cause renal tubular damage

In the next 5 experiments local anesthetic was infiltrated into one renal pedicle during clamping of the aorta In 5 further clamping experiments a ganglion blocking agent was used Xylocaine without epinephrine was used in 2% solution and 4 ml were injected under the adventitia of the renal artery of one side The contralateral renal pedicle was not infiltrated

It was found that renal tubular damage on the infiltrated side was not so conspicuous as in the other kidney

The ganglion blocking agent employed was arfonad This drug was given by intravenous drip in amounts adequate to lower systolic blood pressure to 70 mm Hg This drug was chosen in preference to hexamethonium because it is more easily controlled and its effects after administration is stopped are of short duration It has moreover a direct effect on arteriolar walls as well as on autonomic ganglia [2]

When arfonad was employed during the aortic clamping, 4 out of 5 dogs showed no histological evidence of renal tubular damage, and in the fifth dog damage was minimal

The effects of aortic cross clamping on renal function were next investigated Selkurt [3] pointed out that renal clearance methods were less reliable than direct methods when ischemia of the kidney was present Therefore the response of the kidneys to a large intravenous infusion of 5% dextrose was used as an index of function, and urine was collected from each kidney separately by diverting the ureters to the body surface, or by dividing the bladder into 2 separate pouches

An initial intravenous infusion (500 ml of 5% dextrose solution) given in 30 minutes was used to produce a diuresis which provided a baseline for control purposes

Control outputs of urine from the right and left kidneys were equal in the dogs used † After an interval of a few days to allow for recovery from the operation, the aorta was later clamped with local anesthetic infiltrated into one renal pedicle During this procedure a further 500 ml infusion was given The most frequently observed response from the control side was a suppression of urine formation whilst the aorta was clamped This oliguria was still present some days later when a further infusion was given The urine excretion from the kidney which was infiltrated with xylocaine was usually profuse commonly 4 times that of the control, and in 1 dog 90 times that of the control

Other control experiments showed that infiltrating the renal pedicle with xylocaine without clamping the aorta produced no alteration in urine flows

When arfonad was used, the effects were as follows Arfonad alone without clamping of the aorta usually lowered the urine output in response to intravenous infusion as compared with control values in the same animals When the aorta was clamped a few days later and arfonad was used, the urine output in 4 of the 5 dogs exceeded that in the control experiment—in 1 case being over 5 times as great

It was found, however that clamping the aorta did not always depress urinary excretion as previously reported and estimates of renal blood flow during aortic clamping showed no significant reduction below control values

† One dog showed marked disparity in urine flows from the two kidneys and was excluded from the experiment In this 1 dog one kidney was found to be rudimentary in size

These experiments showed that the use of local anesthetic to the renal pedicles or of ganglionic blockade in clamping of the abdominal aorta will significantly reduce the degree of histological tubular damage which would otherwise be produced.

Similarly, depression of renal function which is a frequent, but not universal experimental finding, may largely be prevented by either of the agents investigated.

Confirmation that arteriolar spasm was responsible for the histological and functional changes was obtained from further experiments in which opaque material was injected *in vivo* into the renal arteries. In control experiments without clamping of the aorta, the entire renal vascular pattern was rendered visible by the opaque material. Kidneys from animals which had aortic clamping performed showed very little or no injected material in the renal vessels. The pattern of the injected vessels from kidneys where ganglionic blockade or local anesthetic was employed in association with clamping of the aorta was identical with the control series.

Some experience of infiltration of the renal pedicles with local anesthetic during aneurysmectomy in human subjects has been obtained by one of the authors (E. M. N.). It is believed that this may be an effective method of lowering the postoperative incidence of azotemia and depression of renal function in such patients.

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AN IMPROVED TRACHEAL PROSTHESIS *

WILLIAM H MONCRIEF, JR, AND JOSEPH E SALVATORE

Despite intensive effort to define an ideal prosthetic replacement for circumferential, or 'sleeve," resections of the trachea and main stem bronchi, most results have been consistently unsatisfactory from the long term standpoint Previous authors have listed those points that characterize the ideal prosthesis 1) prosthetic tube must be permanent, 2) bridging tube must be placed inside the lumen of the cut trachealends 3) prosthesis must be impervious to granulations growing through the walls, 4) prosthesis must properly fit the severed tracheal ends, both length and diameter, 5) dependable fixation of the prosthesis must be attained [1] To these characteristics we would add 6) the prosthesis must possess rigidity enough not to collapse with inspiration but elasticity enough that it will not erode adjacent cervical or mediastinal structures

The characteristics of elasticity plus rigidity and the strong feeling that the tube must be permanent, and being permanent result in minimal host reaction, led us to seek our answer among the plastics In addition we were desirous of a plastic that could be precast or moulded relatively easily in our own laboratory Keeping these principle qualifications in mind we elected a plastisol of polyvinyl chloride, Tygoflex 75® †

METHOD

The prosthesis. Tygoflex 75® is a liquid plastisol of polyvinyl chloride At temperatures of 350 to 400°F it fuses into a tough, flexible material fully nontoxic and essentially nonreactive Selecting an aluminum die (Fig 1) slightly smaller than the diameter of the host trachea, and with grooves cut at intervals to simulate the tracheal rings and to furnish rigidity to the prosthesis, the liquid plastisol is either "brushed on' the preheated die or the preheated die 'dipped into the plastisol for the length of time that experience has shown will furnish the desired thickness of the prosthesis The plastisol is subsequently fused by the application of heat Using this technique one develops, quickly and inexpensively, an elastic prosthesis with sufficient rigidity to prevent collapsing on inspiration and in a multiplicity of sizes and shapes (Fig 2)

By simply turning the prosthesis inside out after its removal from the die one has a point for fixation of the prosthesis to the trachea Remembering the criterion that "the bridging tube must be placed within the lumen of the

† Trademark United States Stoneware Corp Akron Ohio

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This article does not represent the official position of the United States Army or any element thereof The authors as individual surgeons stand responsible for the opinions expressed



Fig 1 The aluminum die available in varying diameters and also as a bifurcation



Fig 2 After curing and removal from the die the prosthesis is turned inside out making the rings available for fixation



Fig 3 The horizontal mattress suture incorporating the black silk suture

cut tracheal ends the prosthesis is fixed securely with interrupted horizontal mattress sutures of stainless steel wire These sutures are placed so that they perforate the cut ends of the trachea and then the ring of the prosthesis is transfixed without entering the lumen of the prosthesis (Fig 3) The black silk suture is included to prevent the stainless steel suture from cutting through the prosthesis

Experimental application With sterile technique sleeve resections of 1 to 6 cartilaginous rings of the low cervical trachea were carried out through a midline incision in 16 dogs Using the right transpleural approach the same type of resection was carried out in the distal trachea just proximal to the carina in 4 dogs Those animals having a transpleural approach had under water seal drainage of the pleural space for 48 hours postoperative and all animals were on penicillin and streptomycin intramuscularly for 5 days post operatively Animals were bronchoscoped immediately postoperatively to insure satisfactory tracheobronchial toilet and then at monthly intervals In 3 animals cervical prostheses were inserted with the plan that these animals would be sacrificed at 3 month intervals and tensile strength studies carried out on the prosthetic to evaluate any changes as compared to the originally inserted material

Two animals have had the distal trachea and main stem bronchi replaced

RESULTS

All animals survived the operative procedure and the immediate post operative period except those that had the tracheal bifurcation replaced In these animals with the carina and main stem bronchi replaced we have not been able to maintain satisfactory pulmonary expansion in spite of bilateral underwater seal drainage with negative pressure and careful postoperative bronchoscopy Tracheostomy and even more careful postoperative care appear to be indicated in this group

In the group operated on for tensile strength studies it became obvious at the time the first animal was sacrificed that we no longer had an elastic prosthesis but a rigid tube This finding will be discussed later

Of the 16 animals that had sleeve resection of the cervical trachea with re placement 10 animals have died Of these 2 were the initial animals operated before the black silk suture was incorporated into the horizontal mattress suture securing the prosthesis All animals that died had displacement of the prosthesis proximally and subsequent stenosis of the fibrous tube replacing

the resected segments of the trachea These animals died from 10 to 24 weeks weeks postoperatively The remainder of the animals are asymptomatic and bronchoscopy in all has revealed the prosthesis in position and well tolerated 8 to 12 months after surgery

Four animals had the distal trachea resected and replaced One of these *animals* was *inadvertently* sacrificed by another investigator 6 months post operatively At autopsy the prosthesis was in perfect position with minimal host reaction The remaining animals are all doing well 8 months postoperatively On bronchoscopy there is no evidence of excessive secretions distal to the prosthesis and the host trachea is without reaction at the site of the anastomosis

DISCUSSION

Our technique of fixation with horizontal mattress sutures of stainless steel without entering the lumen of the prosthesis has proven most satisfactory The technique is easily carried out results in an airtight suture line and is without evidence of stenosis on long term followup

We feel from our studies that the elastic prostheses are to be preferred to the more rigid tubes Our initial enthusiasm for the polyvinyl chloride prosthesis has been tempered somewhat by the finding of the loss of elasticity after 3 months implantation This material is one of a group of polymers that requires additions called plasticizers Polyvinyl chloride itself is a rigid material which requires these additions to produce pliability and elastic qualities The difficulty encountered with this group of polymers is that the plasticizers tend to leach out leaving the original nonpliable rigid substance

There are several routes to be investigated to circumvent the difficulties encountered with these materials Since this plastic has many desirable characteristics it may be combined with another polymer to produce the combined and desirable properties without the use of a plasticizing agent Another answer could be the selection of a polymer which is inherently elastic and requires no plasticizing agent Current efforts are being concentrated in these *directions to determine a more ideal material* for tracheal replacement

SUMMARY

To the established criteria for the ideal tracheal prosthesis we would add the prosthesis must possess enough rigidity so as not to collapse with inspiration but enough elasticity that it will not erode adjacent cervical and mediastinal structures and even give with respirations

A more efficient technique for permanent fixation of the prosthesis within the cut tracheal ends is detailed

A brief characterization of the more ideal material from which to handily fashion the prosthesis and our efforts in that direction are briefly discussed

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EXTENSIVE SEGMENTAL RESECTION OF THE TRACHEA WITH PRIMARY SUTURE ANASTOMOSIS *

An Experimental Study

MAX L SOM AND SAMUEL H KLEIN

The purpose of this report is to present a method by means of which end to end suture anastomosis of the trachea in the dog has been successfully accomplished following resection of a wide segment in one stage The principle involved concerns the requirement that sufficient tracheal elongation be obtained to permit approximation of the cut ends without tension thus preventing disruption of the suture line

The trachea is a relatively inelastic organ in its longitudinal axis by virtue of the fact that the supporting cartilaginous rings are bound together by the fibrous annular ligaments This tubular structure is further fixed at both ends by its attachment above to the larynx and to the pulmonary hilum in the mediastinum below

It occurred to us that it might be possible to stretch the trachea longitudinally if the annular ligaments between the cartilaginous rings were divided circumferentially down to the level of the mucosal layer (Fig 1) The elasticity of this soft tissue and of the posterior membranous wall of the trachea might then permit sufficient separation of the tracheal rings to produce an increase in length of the organ

METHOD

This procedure was first tried on a segment of human trachea obtained at autopsy An appreciable increase in length did occur

It was then attempted *in vivo* in dogs Starting usually at the level of the 6th tracheal ring segments consisting of from 9 to 13 rings were mobilized and then resected It was noted that the cut ends of the trachea could now be brought together only with great tension if at all However approximation could be readily accomplished after incision of the annular ligaments and separation of the tracheal rings Usually 5 annular ligaments to the level of the cricoid cartilage were successively divided in the remaining proximal portion of the trachea Below the distal line of resection, the procedure was carried out as far as possible incising usually 5 to 7 annular ligaments

The most favorable technique of anastomosis proved to be simple suture of the mucosa with eversion by means of interrupted mattress sutures of 4 0 black silk (Fig 1) On occasion 2 or 3 stitches were passed through the edges of the apposing cartilages The use of silk or wire perichondral sutures was found to be unnecessary Splinting of the anastomosis by means of polyethylene tubing fixed in the lumen of the trachea proved to be not only unnecessary but seemed actually to be harmful

The suture line was covered by a narrow strip of gelfoam placed around its circumference On several occasions the mucosa was inadvertently nicked in

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* From the Departments of Otolaryngology and Surgery The Mount Sinai Hospital New York N Y With the assistance of Dr Bernard Wolf Director of the Department of Radiology in the roentgenographic studies and Dr Sadao Otani Attending Pathologist in the microscopic examination of postmortem specimens

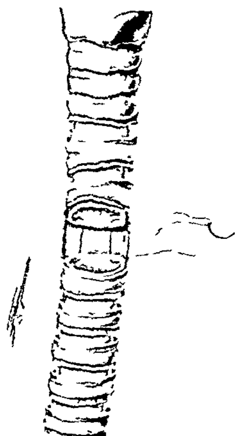


Fig 1 Drawing of trachea showing separation between cartilaginous rings after circumferential incision of annular ligaments down to mucosal layer Note the placement of mucosal everting mattress sutures for anastomosis

one or more places while incising the annular ligaments These small openings were simply covered over by a patch of gelfoam

The operation was concluded with closure of the cervical musculature and the skin without drainage

The procedure was performed in 19 dogs, with the following results 1 dog died shortly after operation, presumably of overdosage of anesthesia One died on the day following operation At autopsy, the intraluminal polyethylene tube was found to have become dislodged upward into the larynx One death occurred on the third day postoperatively Necropsy showed extensive tracheal necrosis, dehiscence of the anastomosis and peritracheal infection This failure was thought to be due to pressure of the intraluminal polyethylene tube and/or interference with the blood supply of the trachea during the operative mobilization A fourth animal died on the 6th day Although the anastomosis was intact, there was no evidence of healing reaction, and the suture line disrupted when handled during the dissection Peritracheal infection was present In this case, postoperative prophylactic penicillin therapy had been inadvertently omitted and may have been a contributing factor in the occurrence of infection

Four dogs died on the 6th, 14th, 15th and 23rd days respectively following operation Pneumonic infiltrations were found in the lungs at postmortem examination The tracheal anastomoses were intact

One animal died on the 37th day At necropsy, it was noted that some separation had taken place at the anastomosis The tracheal wall bridging the gap now consisted of fibrous scar tissue There was moderate narrowing of the lumen, reducing its caliber by about one third Although there was adequate airway at this time, it seems probable that had the ani

mal survived further stricturing would eventually have supervened

In 1 dog bronchoscopy on the 11th day postoperatively found the tracheal lumen to be normal in caliber About 5 weeks later however, respiratory stridor began to be observed and bronchoscopic examination revealed that the trachea had become strictured at the suture line The animal died a few days later (on the 53rd postoperative day) Autopsy examination corroborated the bronchoscopic observation of marked stricture formation

Nine dogs survived the operation without untoward incident and were followed for periods of 11 98 105 119 126 138 225, 229 and 132 days respectively At the end of these intervals the operative areas were examined by bronchoscopy and roentgenography With the exception of the last one the animals were then sacrificed and autopsy performed The last dog was followed further until 2 ½ years had elapsed after operation Bronchoscopy and x ray examination were then repeated following which the dog was sacrificed to permit postmortem study

The roentgen studies included films taken in the anteroposterior and lateral positions The tracheal walls were well delineated by air contrast as well as by the use of instilled radiopaque oil or insufflated barium powder The latter, adhering in a thin film to the moist surface of the mucous membrane, afforded better detail on the films

Moderate circumferential narrowing of the anastomotic site was noted in 1 case and deformity with some narrowing was seen in the lateral roentgenogram in another Neither dog presented any respiratory difficulty however Healing was complete in all these animals and the tracheal lumen with the 2 exceptions noted above appeared to be normal (Fig 2) It was noted further that the spaces between the cartilaginous rings where the annular ligaments had been incised were filled in with fibrous tissue

The suture line was also examined microscopically in 2 of the tracheal



Fig 2 Lateral roentgenogram of trachea made 2¼ years after resection of segment of 13 tracheal rings and primary suture anastomosis Five annular ligaments were incised in the proximal portion of the trachea and 9 ligaments in the distal portion

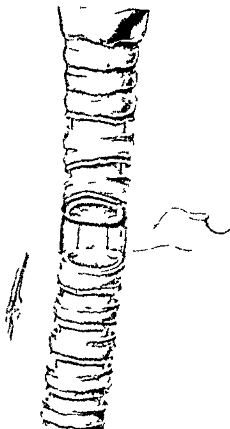


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In 1 dog, bronchoscopy on the 14th day postoperatively found the tracheal lumen to be normal in caliber. About 5 weeks later, however, respiratory stridor began to be observed, and bronchoscopic examination revealed that the trachea had become strictured at the suture line. The animal died a few days later (on the 53rd postoperative day). Autopsy examination corroborated the bronchoscopic observation of marked stricture formation.

Nine dogs survived the operation without untoward incident and were followed for periods of 41, 98, 105, 119, 126, 138, 225, 229 and 132 days respectively. At the end of these intervals, the operative areas were examined by bronchoscopy and roentgenography. With the exception of the last one, the animals were then sacrificed and autopsy performed. The last dog was followed further until 2-½ years had elapsed after operation. Bronchoscopy and x-ray examination were then repeated, following which the dog was sacrificed to permit postmortem study.

The roentgen studies included films taken in the anteroposterior and lateral positions. The tracheal walls were well delineated by air contrast as well as by the use of instilled radiopaque oil or insufflated barium powder. The latter, adhering in a thin film to the moist surface of the mucous membrane, afforded better detail on the films.

Moderate circumferential narrowing of the anastomotic site was noted in 1 case, and deformity with some narrowing was seen in the lateral roentgenogram in another. Neither dog presented any respiratory difficulty, however. Healing was complete in all these animals and the tracheal lumen, with the 2 exceptions noted above, appeared to be normal (Fig. 2). It was noted further that the spaces between the cartilaginous rings where the annular ligaments had been incised were filled in with fibrous tissue.

The suture line was also examined microscopically in 2 of the tracheal



Fig 2 Lateral roentgenogram of trachea made 2½ years after resection of segment of 13 tracheal rings and primary suture anastomosis Five annular ligaments were incised in the proximal portion of the trachea and 9 ligaments in the distal portion

specimens The apposing cartilages were seen to be held in close approximation by partially hyalinized fibrous tissue and the luminal surface of the healed area was covered by growth of a modified low columnar epithelium

CONCLUSIONS

Successful end to end suture anastomosis of the dog s trachea can be accomplished following wide segmental sleeve resection in one stage

Tension at the suture line may be avoided by a method of increasing tracheal length This consists of incision of the intercartilaginous annular ligaments thus permitting the wall of the trachea to be stretched in its longitudinal axis

Technical failures are described

When the occasion arises to use this procedure clinically in the human complementary tracheostomy distal to the anastomosis will be employed and the cervical wound drained to avoid infection

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CHANGES IN PULMONARY VASCULAR LESIONS AFTER RESTORING NORMAL PULMONARY ARTERY PRESSURE *

RICHARD H BLANK WILLIAM H MULLER JR AND
J FRANCIS DAMMANN JR

The question of whether or not the anatomical lesions in the pulmonary vasculature of certain patients with congenital heart disease will regress following surgery has acquired increasing importance in recent years

Dammann Baker and Muller[1] previously described the sequence of development of pulmonary arterial lesions in the left upper lobe of the dog lung which had been exposed to a systemic blood pressure and flow The present study concerns itself with the changes in similarly produced lesions following interruption of the inciting stimulus More specifically it was wished to determine if a point existed at which changes would no longer regress

METHOD

An end to end anastomosis between the subclavian artery and the distal left upper lobe pulmonary artery was constructed in 46 adult mongrel dogs In 14 additional animals the distal left lower lobe pulmonary artery was anastomosed to the side of the aorta or to the end of the subclavian artery At intervals varying from 1 hour to 9 months the anastomosis was interrupted and the lobar pulmonary artery was reanastomosed to the left pulmonary artery in 20 dogs Arterial pressures were recorded in the aorta subclavian and involved pulmonary artery at both procedures in the majority of animals From periods of a few hours to one year after the reversal operation the

* From the Department of Surgical Research University of Virginia School of Medicine Charlottesville Supported by U S P H Grant H 2038

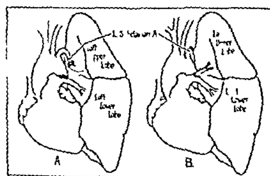
involved lung was biopsied or removed. Microscopic sections were prepared with both elastic tissue and hematoxylin and eosin stains and multiple sectioning techniques were used in those animals severely affected in order to avoid misinterpretation of focal changes.

A comparison was then made between control sections, biopsies obtained at the "reversal" operation and the final specimens obtained in each animal. Objective evidence of healing occurring between the "reversal" operation and the time of the final biopsy was determined by several methods. Changes in the walls and lumens of the pulmonary arteries in the various sections of each animal having advanced lesions were measured with a micrometer eye piece and the lumen wall ratios* calculated. The difference in the total number of obstructed arteries per lower power field in the pre and postreversal sections was also used as an index of regression.

RESULTS

Forty per cent of the animals succumbed in the early postoperative period. The majority of deaths were attributable to pulmonary edema. The anastomosis was found to be thrombosed in 6 animals at the time that the second biopsy was obtained. At the operation for final biopsy, the reanastomotic suture line was found to be patent in 4 animals and thrombosed in 7 but was not examined in 2 animals. In the animals with thrombosis of the reversal anastomosis, the distal lobar pulmonary artery was found to be patent in each case either by demonstrating it with an angiogram or actually dissecting it free for several centimeters into the lung parenchyma and inspecting it.

The development of the arterial changes was similar to the sequence described previously.[1] It was observed that the subclavian left upper lobe artery shunt produced the most consistent vascular lesions. Those animals having the lower lobe pulmonary artery anastomosed to a systemic vessel did not develop advanced lesions.



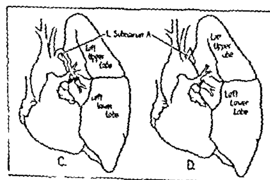


Fig. 1. Top: (a) Subclavian left upper lobe pulmonary arterial anastomosis. (b) Reversed.

Bottom: (c) Subclavian left lower lobe pulmonary arterial anastomosis. (d) Reversed.

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METHOD

An end to end anastomosis between the subclavian artery and the distal left upper lobe pulmonary artery was constructed in 16 adult mongrel dogs In 11 additional animals the distal left lower lobe pulmonary artery was anastomosed to the side of the aorta or to the end of the subclavian artery At intervals varying from 1 hour to 9 months the anastomosis was interrupted and the lobar pulmonary artery was reanastomosed to the left pulmonary artery in 20 dogs Arterial pressures were recorded in the aorta subclavian, and involved pulmonary artery at both procedures in the majority of animals From periods of a few hours to one year after the reversal operation, the

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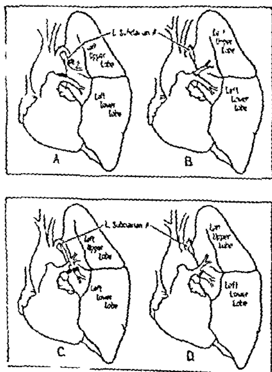


Fig 1 Top (a) Subclavian left upper lobe pulmonary arterial anastomosis (b) 'Reversed

Bottom (c) Subclavian left lower lobe pulmonary arterial anastomosis (d) 'Reversed

The arterial pressures on the pulmonary side of the anastomosis generally correlated with the vascular alterations which subsequently developed If the pulmonary artery pressure did not approximate systemic levels, the vascular changes were usually minimal There was frequently an increase in the lobar pulmonary artery pressure between the first and second operations, which was interpreted as representing an increased resistance to blood flow through the involved lobe

Thirteen animals are available for study at the present time In 2 in which the systemic pulmonary artery shunt was interrupted at the end of 1 hour and 1 week respectively, the hemorrhagic pneumonitis and perivasculitis demonstrated significant clearing by the end of 3 months Increased perivascular adventitial tissue was noticeable about the small muscular arteries and arterioles at the end of this time however, but the luminal diameters did not appear to be reduced

In 5 animals, in which reversal after longer intervals was performed minimal to moderate degrees of hypertrophy of the medial layers of the muscular arteries and arterioles occurred The degree of healing occurring between the second and third operations in this group was difficult to assess In most instances the wall thickness of the involved arteries appeared to be reduced although perivascular fibrosis was usually present in the final sections Progression of the vascular lesions was not observed following interruption of the hypertensive stimulus

Six animals had developed advanced pulmonary arterial lesions consisting of varying degrees of both medial hypertrophy and intimal hyperplasia (Fig 2a) Examination of microscopic sections obtained from several hours to 4 months following the reversal operation revealed noticeable increased luminal diameters of the involved vessels as early as 11 days While the majority of vessels still bore evidence of injury as reflected by the fragmented elastic lamina and intimal fibrosis (Fig 2b), there appeared to be a significant increase in the over all cross sectional diameter of available vascular pathways This was confirmed by the lumen wall ratio determinations and objective counting of the number of totally obliterated arteries in the pre and post reversal biopsy material

Realizing that functional studies are of importance in such a study, an angiogram of the pulmonary vascular bed at the second operation has been obtained and correlated with another obtained later during the healing period The initial results with this technique have supported the impressions gained by histological study alone

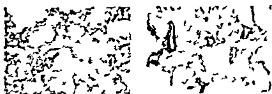


Fig 2 (a) Dog 227 Peripheral lung biopsy (× 70) obtained at the "reversal operation" 4 months following establishment of a subclavian left upper lobe pulmonary arterial anastomosis Notice the degree of luminal occlusion caused by proliferative intimal changes in the pulmonary arteries

(b) Dog 227 Biopsy (× 70) obtained from the same area of involved lung 3 months after the "reversal" operation While evidence of vascular injury is still present the over all cross sectional diameter of the pulmonary arteries is significantly increased

DISCUSSION

Ferguson and his associates[3, 4] have studied the effects of removing systemic pulmonary arterial shunts from various lobes of the dog lung. They concluded that in those animals having only medial hypertrophy of the pulmonary arteries, significant regression did not occur during time periods equal to those required for the lesions to develop In addition, they observed that in those animals having both medial hypertrophy and intimal hyperplasia, vascular patency occurred at a slow rate following removal of the provoking stimulus

The present study differs from that of Ferguson in that we have utilized a smaller volume of lung tissue as the target vascular bed. The discrepancy in the fate of advanced arterial lesions in the two experiments is difficult to explain. Initially, we thought that perhaps the smaller lung volume used in this study might result in excessive initial trauma to the vascular bed and that the resulting lesions merely represented the residua of acute vascular injury. It became apparent, however, that vascular changes occurring in animals reversed shortly after establishment of the shunt, healed rapidly with no evidence of progression. In addition, a study of the lungs of 26 animals at periods from a few hours to 4 months following establishment of systemic pulmonary arterial shunts revealed that an orderly pattern existed in the development of vascular lesions. In brief, the appearance of a medial layer in vessels of arteriolar dimensions is the first observed change. Medial hypertrophy of the muscular arteries followed by intimal hyperplasia of both the arteries and arterioles completes the cycle of development.

The apparent early attempts at healing in even the most severely affected pulmonary vascular beds in this study is encouraging. The question arises, however, as to whether a severely diseased vascular bed will be able to heal in the presence of higher blood flows and pressures than produced in this experiment This will be the case clinically since closure of an intercirculatory communication in the presence of marked pulmonary hypertension places an equal or greater burden on the lesser circulation It is for this reason that we are presently seeking other experimental preparations that more closely simulate conditions as they occur in man

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THE DEVELOPMENT OF CHRONIC PULMONARY HYPERTENSION *

C. FREDERICK KITTLE, LUIS BIANCHINI, THEODORE HOSTETLER AND WILLIAM A. REED

The experimental production of pulmonary hypertension has been of interest to many investigators because of its implications regarding the basic anatomy and physiology of pulmonary hypertension and pulmonary emboli as well as their clinical manifestations.[1] We have recently attempted to develop a method of chronic pulmonary hypertension to produce regional or local hypoxemia to various organs or areas of the body. By anastomosing the pulmonary artery to a systemic artery, that particular area supplied by the systemic artery *would then be perfused by venous blood if the perfusion pressure* were sufficient.

Previous methods for the production of pulmonary hypertension have been chiefly concerned with either a systemic pulmonary vascular shunt or obstruction of the pulmonary venous outflow tracts.[2] Numerous substances have been used for pulmonary embolization, but generally pulmonary pressures have not been measured either in the immediate or the late postinjection period.

METHOD

Fourteen mongrel dogs between 11 and 16 kg. constituted the *final group* for these data. Each animal was injected via the external jugular vein generally 3 times weekly with either 0.5 or 1.0 gm. of carborundum particles. This dosage was selected after observing that a larger amount was not tolerated and generally resulted in death from acute pulmonary embolism as manifested by dyspnea, frothing at the mouth, tachycardia and hypotension.

The injection was done through a 15 needle, flushing the carborundum particles with approximately 150 to 300 ml. of normal saline solution. This maintained an even and fairly uniform dispersion of the particulate matter throughout the 5 to 10 minute period of injection. It also assured the carborundum particles of being carried to the lungs rather than being deposited as sediment in the external jugular, the innominate, or the superior vena cava.

The size of the carborundum particles varied from 50 to 400 μ (*corresponding to grit size 60 to 180*). *Injections were made 3 times weekly* in most animals until the desired dosage had been reached. Right heart catheterization was then done at varied intervals after discontinuing the injections. The animals were lightly anesthetized with sodium pentobarbital. Right ventricular and pulmonary artery pressures were recorded.

None of the animals died because of the injections although the total dose in each instance was many times the immediate lethal dose. When electively sacrificed 21 to 58 weeks after the initial injection, x-rays were taken of the lungs. Subsequently the lungs were digested and the amount of carborundum *actually present in the lungs determined. Examination of the heart was made* for evidence of right ventricular hypertrophy and to note whether or not all the carborundum particles had passed into the pulmonary vessels.

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* From the Department of Surgery, University of Kansas Medical Center, Kansas City.
Supported by U. S. P. H. Grant #H [illegible] and the John and Mary R. Markle Fund.

RESULTS

Twenty-four right heart catheterizations were done in these 11 dogs In 11 dogs pulmonary hypertension was present. Normal pulmonary artery pressures had been measured in another group of experiments and were found to range from 18 to 31 systolic and 8 to 18 diastolic with a mean of 27/13 mm. of Hg in 21 observations in 21 animals These are essentially the same pulmonary pressures as noted by others[3] Any systolic pressure over 35 was arbitrarily defined as pulmonary hypertension.

Pressures of 70 mm. Hg systolic were measured in 2 animals and in 7 animals pressures from 35 to 70 mm. systolic were found. These pressures were not sustained over a prolonged period as evidenced by repeat catheterizations in the same animal when a gradual diminution in pulmonary pressure occurred

When the animals were electively sacrificed at various intervals after catheterization the carborundum particles were found uniformly distributed throughout all lobes of the lung within the pulmonary vessels from the hilum to a subpleural location In the larger vessels the particles had clumped together to form localized aggregates In other locations, particularly subpleural, the particles remained discrete and occasionally surrounded by fibrous tissue (Fig. 1) Roentgenograms of these specimens visualized well the vascular pattern of the lung with fairly even dispersion throughout all lobes (Figs 2, 3, 4)

In 9 of the 11 specimens the lungs were digested with warm 20% potassium hydroxide and the carborundum recovered. A minimum of 85% of the injected dose was found in these lungs Grossly it had been noted that small aggregates of carborundum were occasionally present in the jugular and innominate veins, the superior vena cava, and along the leaflets and chordae tendinae of the tricuspid valve The right ventricular wall varied from 0 5 to 1 0 cm in thickness suggesting slight ventricular hypertrophy.



Fig 1 Gross specimen of dog 121 (15 kg dog receiving 26 gm of 60 grit size carborundum) *A* illustrates the subpleural location of these particles and *B* the fairly uniform dispersion throughout the cut surface of the lung



Fig 2 Roentgenogram of lung from dog 121 Note delineation of the vascular pattern of the lung with the uniform distribution to all lobes



Fig 3 Roentgenogram of lung from dog 141 (12 7 kg dog given 34 gm of 60 grit size carborundum) Pulmonary hypertension (44/24) present 12 months after discontinuing injections

DISCUSSION

The occurrence of pulmonary hypertension after obstruction of the pulmonary vessels is a direct consequence of increased pulmonary resistance In our particular type of preparation the pulmonary vessels receive the same amount of unoxygenated blood from the right ventricle but obstruction to flow results in a greater pulmonary pressure an increased right ventricular pressure and subsequent right ventricular hypertrophy

The immediate development of increased pulmonary artery pressure after pulmonary embolization has been noted experimentally and clinically but it is not agreed whether this is due to anatomic obstruction *per se* or whether an associated vasospasm occurs That the pressure decreases in time suggests that vasospasm may be initially present or that vascular channels are opened which previously had not functioned When one considers that a dose of 1 5 or 2 0 gm of the carborundum particles used here is generally fatal the ability of the pulmonary vascular bed to contain as much as 34 gm without mortality suggests that vasospasm may be partly responsible for death in acute embolization

The gradual decline of pulmonary hypertension as shown by successive catheterizations in several dogs (39 106 107 131 and 141 see Table 1) illustrates the voluminous capacity of the pulmonary vascular bed and its gradual expansion to reduce pulmonary resistance and work of the right ventricle

SUMMARY

1 A method for the production of pulmonary hypertension in dogs by repeated injections of particulate matter (carborundum particles 50 to 100 μ) intravenously is described

2 Pulmonary hypertension when established may persist for several months but repeat measurements show a gradual decline in the pulmonary artery pressure

3 The amount of particulate matter required to produce chronic pulmonary hypertension is many times the acute lethal dose

Table 1

DOG	WT KG	NO. INJECTIONS	SIZE CARB	WT CARB	PULMONARY ARTERY PRESSURE — MONTHS AFTER LAST INJECTION 1	2	3	4	5	6	7	8	9	10	11	12	13
39	15.7	20	1	17.5	60/40	58/35	54/40			11/25							33/ †
101	15.8	11	1	11.5	25/12												
106	11.9	12	2	13.0	70/30	30/10											
107	11.5	11	1	16.3	70/30	60/25		60/41									
121	15.0	31	1	26.0				37/20									
131	11.0	36	1	32.0	52/34			37/20									
131	15.1	16	1	16.0	50/30												
137	11.8	31	1	30.5	45/15												
138	12.8	31	1	31.0	32/10												
111	12.7	10	1	31.0				54/24							11/21		
202	15.0	19	1	11.0				28/12							41/ †		
205	15.6	17	1	9.0				14/28									
212	11.5	18	1	11.0				38/26									
213	15.3	17	1 ††	10.5				28/12									

† Right ventricular pressures

†† Size 1 is grit 60 (300 to 400 μ), size 2, grit #80 (200 to 300 μ) size 4, grit #180 (50 to 90 μ)

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OBSERVATIONS ON THE EFFECT OF NORETHANDROLONE IN FAVORING ANABOLISM IN PATIENTS UNDERGOING PULMONARY RESECTION FOR TUBERCULOSIS

JOHN HINES KENNEDY, HAROLD A PETERS AND GEORGE S SERIF

It has been well established by other investigators[1 2 3] that after any severe trauma including a planned operative procedure the rate of protein break down is greatly accelerated That this catobolic effect can be reversed by the use of testosterone and its derivatives has been demonstrated[4 5] One such synthetic steroid which has been the subject of recent interest is Norethan drolone† (17 ethyl 17 hydroxy 19 nor 4 androsten 3 one) which is strongly anabolic but has few of the androgenic properties of testosterone Individuals undergoing resectional surgery for pulmonary tuberculosis represent a group of patients subjected to severe surgical trauma in the presence of a chronic infection with its concomitant chronic stress reaction This report deals with *work still in progress its purpose is to record the influence* of Norethan drolone on the nitrogen balance of patients undergoing pulmonary resection for tuberculosis

METHOD

Eleven clinically well nourished patients without complicating medical disorders other than pulmonary tuberculosis who were scheduled to undergo elective pulmonary resection were selected for study and randomized into 2 groups After a preoperative control balance period of 3 days the patients in the study group received Norethandrolone (Nilevar®) 50 mg by intramuscu lar injection daily through the 12th postoperative day The patients in the control group received a placebo The total period of observation was 15 days Patients with all degrees of severity of pulmonary tuberculosis who *had achieved sputum conversion on antituberculous chemotherapy* pre operatively were included in the study Excluded from the study were patients with extrathoracic tuberculosis complicating medical disorders other than tuberculosis and those patients whose balance studies were incomplete because of loss of a specimen or errors in collection

† Supplied by G D Searle and Company Chicago

From the San Diego County Hospital Supported by a Grant from the San Diego County Tuberculosis and Health Association

All patients were offered a 2500 calorie high protein diet and analysis of the uneaten diet for total nitrogen was used as a means of arriving at an accurate estimate of total nitrogen intake An estimate of nitrogen balance was calculated by comparing the total nitrogen excretion in pooled homogenized 3 day collections of pleural fluid urine and feces as compared to the total nitrogen intake in pooled homogenized 3 day duplicate diet specimens Collections were made in tared containers and preserved in the frozen state until analysis

Aliquots of pooled 3 day intake and output specimens to which had been added an antifoam preparation were analyzed for total nitrogen by digestion and nesslerization Nitrogen analyses were completed by comparison with a standard nitrogen solution using a Beckman spectrophotometer Model B

OBSERVATIONS

Six of 11 patients were in negative nitrogen balance in their preoperative period before the study was initiated The mean preoperative nitrogen balance for the entire series was minus 2 1 gm for the 3 day preoperative balance period The nitrogen balances of 7 patients who received Norethandrolone are recorded in Figure 1 It will be noted that at no time did the mean nitrogen balance indicate a return to positive nitrogen balance A calculation of the standard deviation from these mean values is recorded Recorded in Figure 2 are the nitrogen balances of 4 patients who received a placebo The patients in the control group had a mean nitrogen balance of minus 16 58 gm for the last 3 day period In Figure 3 a graphic comparison of the mean nitrogen balances for the control and Norethandrolone group are recorded Despite the initial impression that the patients in the control group were in more severe negative nitrogen balance at the conclusion of the study a calculation of the standard error of the difference between two means suggests that this is not a statistically significant difference When this became apparent 1 patient was given twice the recommended daily dose of Norethandrolone and this balance study as recorded in Figure 4 showed a prompt return to positive nitrogen balance

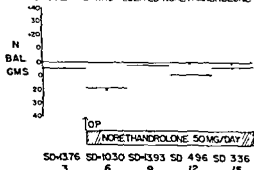


Fig 1 Mean nitrogen balance is indicated by the horizontal black lines

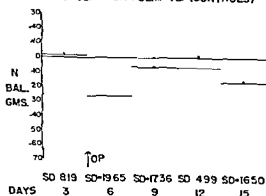


Fig 2 Mean nitrogen balance is indicated by the horizontal black lines

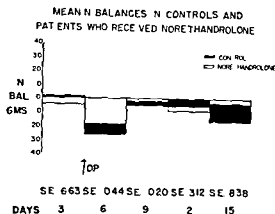


Fig 3 A comparison of the mean nitrogen balances in the control and experimental group The standard error of difference between two means (S E) is recorded

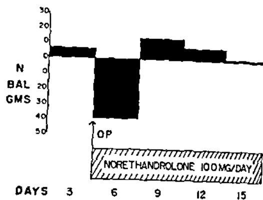


Fig 4 A S a 3 year old Caucasian male who underwent apical and posterior segmentectomy for tuberculosis and who received 100 mg of Norethandrolone/day postoperatively

DISCUSSION

Allowing for the small size of the sample there was no statistically significant difference in the nitrogen balances of patients undergoing pulmonary resection for tuberculosis who received Norethandrolone as compared with the control group employing the recommended dose of 50 mg intramuscularly daily It is of interest that on a dosage program of twice the recommended dose 1 patient showed a prompt return to postive nitrogen balance postoperatively although this single observation cannot be considered of statistical importance It may be that patients subjected to the chronic stress situation of pulmonary tuberculosis require a much greater dose of the anabolic agent Norethandrolone when confronted by the additional stress of a surgical procedure if one is to anticipate a return to positive nitrogen balance This continuing study will be carried on using a dose of 100 mg of Norethandrolone intramuscularly daily and the results will be included in a subsequent report

SUMMARY

The authors report the use of a synthetic steroid Norethandrolone (17 ethyl 17 hydroxy 19 nor 4 androsten 3 one) in 7 clinically well nourished patients who underwent pulmonary resection for tuberculosis Patients were selected by random numbers to serve as the experimental group and after a control balance period were given 50 mg/day of Norethandrolone by intramuscular injection for 12 days following surgery Four patients served as controls and received a placebo

All patients coming to surgery although they were in good clinical condition and had achieved sputum conversion prior to surgery were in negative nitrogen balance with a mean of minus 2 1 gm of nitrogen for the preoperative balance period The mean nitrogen balance for the 7 patients who received the Norethandrolone failed to demonstrate the anticipated return toward positive nitrogen balance The 4 patients who served as controls remained in negative nitrogen balance which in many cases was as great as 20 5 gm of nitrogen/day and had not returned to the preoperative level of nitrogen balance at the end of the experimental period 12 days postoperative No

statistically significant difference could be demonstrated between the control and experimental groups

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PULMONARY EDEMA FOLLOWING THE RAPID REEXPANSION OF A TOTALLY COLLAPSED LUNG DUE TO A PNEUMOTHORAX A CLINICAL AND EXPERIMENTAL STUDY *

Robert I Carlson Kenneth L Classen, Frank Gollan
Walter G Gobbel Jr David E Sherman and
Raymond O Christensen

Recently we encountered 5 patients with a spontaneous pneumothorax in whom reexpansion of the lung was delayed by the appearance of a pulmonary infiltrate together with recollapse of the lung after institution of waterseal drainage

An illustrative case is V A H No 59 019 This 31 year old white male was admitted to the Thayer Veterans Administration Hospital on December 27, 1955 with a history of a spontaneous pneumothorax of 5 weeks duration A 20 F intercostal catheter was placed in the right pleural space and was connected to a waterseal trap Despite numerous endotracheal aspirations a bronchoscopy Stedman pump suction and the use of isuprel and antibiotics it was 10 days before this patient s lung reexpanded and the catheter was removed Representative chest x rays are presented in Figure 1

This case stimulated our interest in this peculiar clinical entity and we attempted to reproduce the condition in the experimental animal

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Fig 1

(A) Preaspiration
(B) 12 Hours Postaspiration
(C) 36 Hours Postaspiration
(D) 9 Days Postaspiration

METHOD

Mature stock laboratory rabbits were used. A daily artificial pneumothorax was instituted, the dose ranging from 15 to 30 cc of air until total collapse of the left lung was produced as proved by x-ray. With the exception of 2 animals, 7 to 8 days later the pleural space was emptied of all air possible by means of a polyethylene catheter passed through a short large bore thoracentesis needle connected to a 50 cc syringe. Immediate postaspiration x-rays were obtained and serial films were made until the time of sacrifice.

The animals were sacrificed by decapitation at intervals from 15 minutes to 21 hours after aspiration. The trachea was ligated in the neck, the chest was opened, and the heart and lungs were removed *en bloc*. Gross and microscopic studies were carried out.

Criteria for validity in this experiment were as follows. a) a total pneumothorax substantiated by adequate x ray films, b) complete reexpansion of the lung following aspiration; and c) x-rays of adequate quality to afford interpretation. The animal was considered to be valid pathologically only if the x ray criteria were satisfied and at the time of sacrifice there was no evidence of trauma to the lung nor of pyogenic infection. Although the animals were placed on tetracycline therapy from the beginning of the experiment, some of the animals had evidence of a pyogenic pulmonary infection and these were discarded.

RESULTS

Two animals were not sacrificed until 11 days after aspiration. The serial x ray findings were similar to those seen in the clinical cases, with an early recollapse of the totally reexpanded lung. Pulmonary consolidation appeared in the recollapsed lung. There was a gradual reabsorption of the intrapleural air and complete reexpansion of the lung in from 7 to 10 days. Pressure measurements done on 1 animal on the fifth postaspiration day revealed pressures of −1, −5 mm. Hg. The serial x-rays of 1 of these animals are shown in Figure 2.

Twenty-seven animals had x ray evidence of a total pneumothorax ranging from 6 to 27 days prior to aspiration. Twenty-one of this group satisfied the x ray criteria. Of these 21 rabbits, 17 showed recollapse of the reexpanded lung and 1 showed evidence only of a shift of the mediastinum to the aspirated side.

Of the 27 animals in the experiment, 11 were deemed to be valid pathologically. Of these 11 valid animals, 8 showed recollapse and 3 showed atelectasis only.

Intrapleural pressure measurements made in 14 animals showed a range of from −1, −2 mm Hg to +20, +30 mm Hg. Of the 5 animals whose intrapleural pressures prior to aspiration were atmospheric or less, 3 showed evidence of recollapse. Of the 9 animals with a positive intrapleural pressure prior to aspiration, all 9 showed evidence of recollapse following aspiration.

Pathological examination was of considerable interest in that the major bronchi and bronchioles were patent and free of obstruction both grossly and microscopically. The histological alterations were well correlated with the radiological changes. Within 15 minutes of aspiration the secondary collapse was manifested by gross and microscopic atelectasis, without edema or cellular infiltration of the alveoli. Lungs of animals sacrificed at varying periods of time up to 18 hours after aspiration also exhibited gross and microscopic diffuse atelectasis. Intraalveolar edema was generally scanty; however, 1 animal sacrificed at 5 hours and another at 18 hours showed an extensive abundant, hyalin, protein coagulum in the alveoli without evidence of inflammatory cell infiltration.

DISCUSSION

Ortner[1] in 1899 reported acute pulmonary edema following thoracentesis in 2 patients with a massive pleural effusion. He states that as early as 1875

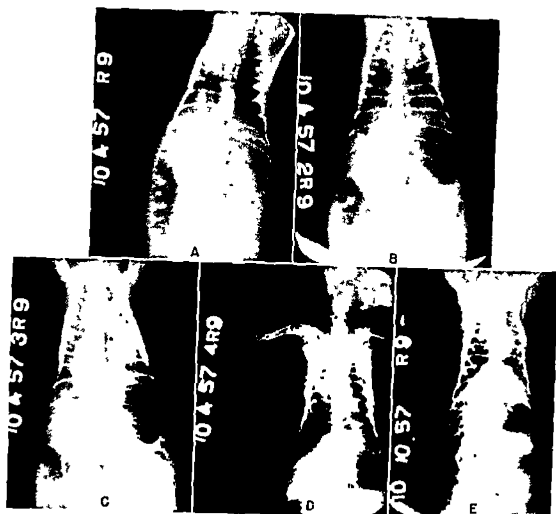


Fig 2

(A) Preaspiration
(B) Postaspiration
(C) 1 Hour Postaspiration
(D) 4 Hours Postaspiration
(E) 6 Days Postaspiration

a Frenchman, Foucart [2] described this phenomenon and Ortner agreed with Foucart that the pathogenesis of the pulmonary edema was probably due to anoxia of the endothelial cells of the pulmonary capillaries On re expansion of the lung following thoracentesis, the restoration of pulmonary circulation was accompanied by the leakage of plasma protein into the alveolar spaces as edema fluid due to the increased capillary permeability Drinker [3,4,5] in this country also felt strongly that anoxia contributed to the formation of pulmonary edema

We have found that the pulmonary edema did not necessarily occur immediately following reexpansion of the lung with presumed restoration of its circulation The formation of pulmonary edema appears to be based on a more complicated mechanism than previously conceived We feel that the edema is probably the result of the atelectasis and recollapse of the pulmonary parenchyma The cause of the underlying atelectasis and recollapse is still obscure

Further studies are being carried out on this problem to determine the possible significance of a physical phenomenon which could cause the

atelectasis Additional studies[6, 7] using cortisone and atropine in an attempt to protect the animal are also under way.

Examination of the lungs of rabbits in which the clinical phenomenon has been reproduced shows no bronchial obstruction; the site of obstruction is intra alveolar. This experimental observation and our clinical experience warrant the conclusion that bronchial aspiration and bronchoscopy are not indicated This self limited phenomenon requires from 5 to 7 days to resolve.

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PULMONARY HEMODYNAMICS AS AFFECTED BY VARYING LEVELS OF RESPIRATORY PRESSURE *

WATTS R WEBB, AND JOSEF R SMITH

Many authors have demonstrated an increase in pulmonary vascular resistance and diminished pulmonary flow during the inflation stage of positive pressure respiration[1] Maloney[2] attributed the effect of pressure to that on the great veins rather than on the pulmonary vessels However, Hubay and his co workers[3] were able to separate the effect on the great veins from that on the pulmonary vasculature They demonstrated that the rise in vascular resistance and fall in pulmonary arterial flow were due primarily to the effect of positive pressure respiration on the pulmonary vasculature itself rather than on the great veins of the chest The study by Weil, Fowler and Makato[4] suggested that overdistention of one lung with 20 cm pressure did not cause a compression of the alveolar capillaries because this lung showed an increased oxygen uptake This study, however, is subject to the criticism that the opposite lung is not protected from the compressing effect of this pressure, as a decrease of arterial oxygen saturation occurred in 4 of 6 dogs during distention of one lung The following experiments were performed to clarify the pulmonary hemodynamics resultant from respiratory pressures, and in particular to determine whether the site of resistance caused by positive pressure is in the alveolar capillary bed

METHOD

Healthy mongrel dogs were anesthetized with pentobarbital, intubated with an endotracheal tube with an inflatable cuff and maintained on

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* From Departments of Surgery and Medicine, University of Mississippi Medical Center, Jackson Aided by National Institutes of Health Grant Number H 2806(C)

mechanical respiration. The left chest was opened and plastic catheters inserted into the pulmonary artery, pulmonary arterial wedge, pulmonary venous wedge and left atrium, for recording of continuous pressures with Statham transducers and a Sanborn multichannel recorder. In addition the pressures were recorded from the pleural space and the tracheal airway. With the chest open, pressures were recorded with intermittent positive and positive negative respiration, and with continuous positive inflation. After the chest was closed, similar observations were made utilizing varying levels of positive and positive negative respiration and spontaneous respiration.

RESULTS

In the open chest the pulmonary venous wedge pressures and the pulmonary arterial pressures showed parallel changes corresponding with the tracheal pressure changes, whether the respirations were intermittently positive or positive negative. These changes appeared to be directly proportional to the tracheal pressure changes. Only on very high sustained pressure did these two separate. At 20 to 25 mm. of Hg the pulmonary artery and pulmonary arterial wedge remained elevated, while the pulmonary venous wedge and the left atrial pressures began to fall, suggesting complete or almost complete alveolar capillary block.

In the low ranges of pressure in the open chest the pulmonary arterial wedge somewhat approximated the left atrial pressures, but these pressures diverged markedly with increasing airway or pleural pressures. The form of these curves was completely different, showing different affecting influences.

A most interesting phenomenon was a drop of pulmonary arterial wedge pressure usually seen at the institution of positive pressure respiration with the chest open and frequently with the chest closed. This phase could easily be missed if the respiratory pressure changes were applied rapidly or if spontaneous respiration was rapid or labored. With further increases of positive pressure the pulmonary artery wedge again showed a typical exponential rise in pressure, though it never reached the pulmonary arterial pressure.

All findings in the closed chest, when the pulmonary vascular pressure changes are plotted against the pleural or tracheal pressure changes with or without the use of a respirator, were very similar to those found in the open chest during relatively low pressure breathing. During prolonged high airway or pleural pressures with the chest closed, however, the delayed fall in pressures of the pulmonary venous wedge and left atrium seen in the open chest was not observed, presumably because the entire thoracic contents were compressed.

DISCUSSION

The above observations point to a definite site of vascular obstruction at the alveolar capillary bed. As anticipated, during quiet respiration the pulmonary venous wedge roughly approximates the pulmonary arterial vascular changes and the pulmonary arterial wedge less closely approximates the changes in the left atrium. Thus our results in general confirm those of Wilson, Hoseth and Dempsey but do show that these relationships are not precise, particularly under abnormal circumstances, and vary independently with the phase and character of respiration.

The initial fall in the pulmonary arterial wedge pressure with slow, gentle positive pressure probably can be explained by the initial thrust of pressure

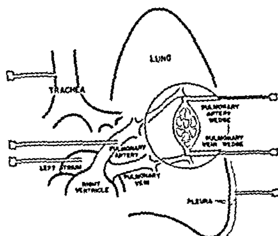


Fig 1 Diagram of pressure recording sites

being against the bronchioles, with straightening and lengthening of the pulmonary radicals and vasculature enlarging the capillary bed. With further increases of airway pressure, the effect becomes transmitted to the alveolar capillary level, with resultant elevation in the pulmonary arterial wedge pressures The initial rise in pulmonary venous wedge and left atrial pressures can be explained by a squeezing effect on the capillary bed, temporarily increasing the flow to the left atrium As anticipated, however, with sustained

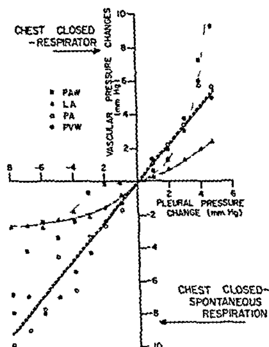


Fig 2 Composite graph showing pressure changes with chest closed on spontaneous and intermittent positive respiration PAW—Pulmonary Artery Wedge LA—Left Atrium PA—Pulmonary Artery and PVW—Pulmonary Venous Wedge Note the parallel changes of the PA and PVW at these relatively low pressures Note the marked divergent trend of the PAW and LA as pressures increase

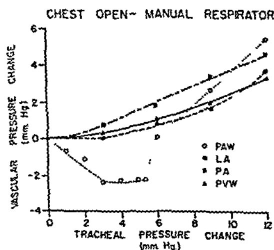


Fig 3 Graph plotting vascular pressure changes against tracheal pressure changes during slow gentle, manual respiration with the chest open Note the drop in PAW pressure during the early increases of airway pressure With higher pressures it rises steeply

high positive pressures this effect dwindles and the pulmonary venous wedge and left atrial pressures fall

The dissociation of the pulmonary arterial wedge and the pulmonary venous wedge with the pulmonary arterial wedge rising much more rapidly indicates the region of block to be at the alveolar capillary bed This effect would appear to be primary in the open chest, while in the closed chest the effect of varying pressures on venous return is likewise a contributing factor to diminishing blood flow through the lungs

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THE EFFECTS OF REDUCED PULMONARY BLOOD FLOW ON PULMONARY CIRCULATION AND RESPIRATORY GAS EXCHANGE *

PAUL H GERST, CHRISTEN RATTENBORG, AND DUNCAN A HOLADAY

It is known that the pulmonary circulation can accommodate a marked increase in blood flow with little alteration in the pulmonary arterial blood pressure, presumably owing to expansion of the pulmonary vascular bed Only fragmentary data, however, are available regarding the response of the pulmonary circulation to *decrease* in blood flow, as in hemorrhagic shock, or after diversion of blood around the lungs as in open heart surgery The results of the present study contribute information on the response of the pulmonary blood vessels to a reduction in pulmonary blood flow and its effects on the respiratory gas exchange

METHOD

Eight dogs were used in this study They were anesthetized with pentobarbital and infused with *succinylcholine* to arrest respiratory muscular activity The animals were ventilated with room air at a uniform rate by a specially designed respirator which delivered a constant tidal volume in the form of a square wave of known duration and volume flow By measuring the *endotracheal airway pressure* a pressure volume curve for each inflation was thus obtained the slope of which directly reflected the total lung thorax compliance [1]

Catheters were placed in the main pulmonary artery and one femoral

* From the Departments of Surgery and Anesthesiology College of *Physicians and* Surgeons Columbia University and the Surgical and Anesthesiology Services The Presbyterian Hospital New York Aided by Grant #H 2[illegible]06 from the National Heart Institute of the U S Public Health Service and a Medical Research Grant from the National Tuberculosis Association through its Medical Section the American Trudeau Society

artery for the collection of blood samples and the simultaneous measurement of pulmonary and systemic arterial blood pressures. At selected intervals we simultaneously obtained samples of arterial and mixed venous blood and end tidal and mixed expired gases. The blood samples were analyzed for oxygen and carbon dioxide contents, oxygen saturation, pH and hematocrit, the gas samples were analyzed for oxygen and carbon dioxide concentrations. From these data we calculated the following: 1) blood carbon dioxide tension and buffer base concentration by the Singer-Hastings nomogram,[2] 2) pulmonary blood flow by the Fick principle; 3) respiratory dead space by the Bohr formula, substituting arterial for alveolar carbon dioxide tension,[3] 4) percentage venous admixture from the arterial and mixed venous blood oxygen contents.[4]

After a 30 minute equilibration period, two sets of control blood and gas samples were obtained one hour apart. We then bled the animals from the other femoral artery in successive steps of approximately 5% of the estimated blood volume, until the systemic blood pressure fell to between 25 to 50% of the control level. A sustained hypotension was obtained after an average of one third of the estimated blood volume had been removed. We maintained the hypotension for one hour during which 3 more sets of blood and gas samples were collected for analyses. Thereafter, the blood was restored and 2 more complete sets of samples were obtained during the following hour.

RESULTS

Blood Pressures. The systemic blood pressure declined continuously during hemorrhage. The pulmonary pressure, however, after an initial decline, stabilized at approximately two thirds of its control level, thereafter falling only slightly. When the blood was replaced, the pulmonary blood pressure *exceeded* its control level, whereas the systemic pressure did not return completely to its prehemorrhagic value (Fig. 1).

Blood Flow. Following hemorrhage, the cardiac output was reduced to between 21 to 35% of control and returned to 68% of control after blood restoration (Fig. 2).

Respiratory Dead Space. The respiratory dead space consists of the sum of the anatomical and the "alveolar" dead spaces.[5] Anatomical dead space is equivalent to the volume of the respiratory passages leading to the alveoli, and "alveolar" dead space is equivalent to the volume of ventilated alveoli which are either inadequately perfused or not perfused at all. Normally, the ventilated alveoli are well perfused so that there is no significant "alveolar" dead space.[6] The carbon dioxide tensions of the end pulmonary capillary blood when measured as arterial blood, and the alveolar gas, when measured as end tidal gas, are then in equilibrium.[3, 4, 7] This we found in our control determinations. If the blood flow to the ventilated alveoli was uniformly reduced throughout the lung, as by partial vasoconstriction, an "alveolar" dead space may develop, but the carbon dioxide tensions of arterial blood and end tidal gas would still be in equilibrium, owing to the great diffusibility of this gas. If, however, blood flow was nonuniformly reduced, as by complete closure of pulmonary blood vessels to some ventilated alveoli, an "alveolar" dead space may develop which would be associated with a carbon dioxide tension difference between end tidal gas and arterial blood.[8]

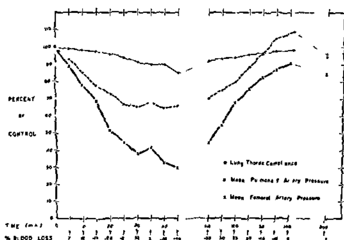


Fig 1. Relative changes in average mean systemic and pulmonary arterial blood pressures and total lung thorax compliance during hemorrhage and during blood replacement The mean values for the control period are pulmonary artery blood pressure, 13 mm Hg, femoral artery blood pressure, 135 mm Hg, lung thorax compliance, 30 5 ml air/mm Hg

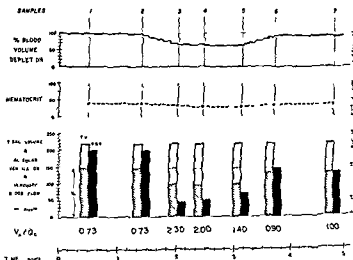


Fig 2 Changes in pulmonary blood flow per inflation, Q_c, (black columns) and tidal volume during control period (1, 2), following hemorrhage (3, 4, 5) and after blood restoration (6, 7) The tidal volume is divided into alveolar ventilation, V_A, (hatched area) and total respiratory dead space (white area) The dead space is further divided by the broken line into anatomical dead space above, and "alveolar" dead space below

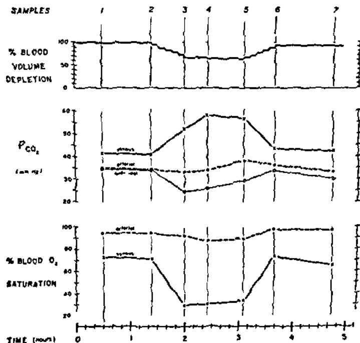


Fig 3 Variations in arterial and mixed venous blood and end tidal gas carbon dioxide tension, and arterial and mixed venous blood oxygen saturation during the control period (1 2) following hemorrhage (3 4, 5) and after blood restoration (6, 7) Note the arterial to end tidal carbon dioxide tension gradient which developed after hemorrhage

Following removal of one third of the estimated blood volume we found that the dead space was augmented by an "alveolar" dead space equivalent to 22% of the tidal volume (Fig 2) and that an arterial to end tidal carbon dioxide tension difference of 8 mm Hg developed (Fig 3) If one assumes that ventilation is uniform throughout the lung these results indicate that a large portion of the previously functioning lung volume was no longer being perfused

DISCUSSION

The two important findings are (a) the maintenance of the pulmonary arterial blood pressure in the face of a markedly reduced pulmonary blood flow and (b) the concomitant development of a significant "alveolar" dead space

The maintenance of the pulmonary blood pressure indicates either a rise in left atrial pressure or a progressive increase in pulmonary vascular resistance To evaluate the first possibility we measured left atrial pressure by direct needle puncture in 2 additional dogs and found a progressive *decrease* during hemorrhage Thus we conclude that as pulmonary blood flow diminishes the pulmonary vascular resistance rises and this process occurs more rapidly in the pulmonary than in the systemic circulation This could occur either owing to progressive and uniform vasoconstriction throughout the lungs or complete closure of an ever increasing number of vascular channels Although an "alveolar" dead space may develop with either mechanism a carbon dioxide tension difference between arterial blood and end tidal gas would occur *only* if some ventilated alveoli were no longer perfused as would result from complete closure of portions of the pulmonary capillary bed

The mechanism by which such nonuniform closure of blood vessels could occur involves the relationship between the forces which tend to keep the vessels open and those which tend to close them The intravascular blood pressure which distends the vessels is opposed by the tension in the vessel walls the surrounding tissue pressure and in the lung the intrapulmonic airway pressure which acts to compress the delicate pulmonary capillaries suspended in an air matrix with little solid support

As pulmonary blood flow decreases there is initially a fall in the intravascular blood pressure When the blood pressure in any portion of the lung drops to a critical level below the opposing pressures the vessels may then close completely[9, 10] This would reduce the size of the vascular bed increase vascular resistance and lead to the maintenance of the central pulmonary blood pressure in spite of progressively decreasing blood flow (Fig 1) When blood flow again increases the resistance offered by these closed vessels to passive reopening would initially require a driving force in excess of that necessary to maintain flow in patent channels This force must be provided by increased work on the part of the right ventricle leading to a rise in pulmonary blood pressure above the control value (Fig 1) otherwise the cardiac output would not be maintained at its prehemorrhagic level (Fig 2)

We feel that the evidence presented here indicates that decrease in pulmonary blood flow can lead to complete closure of pulmonary blood vessels at least during intermittent positive pressure ventilation and that following restoration of blood flow there is some delay before these vessels again reopen Such a mechanism may help to clarify some of the respiratory difficulties

occasionally seen in surgical patients in hemorrhagic shock or following complete circulatory bypassing of the lungs as in open heart procedures [11]

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A STUDY OF CIRCULATION IN THE LUNG FOLLOWING PULMONARY ARTERY OCCLUSION *

RICHARD N MOERSCH AND DAVID E DONALD

The increasing extensiveness of thoracic surgical procedures and the growing use of systems of extracorporeal circulation have given urgency to questions regarding the possible dangers inherent in the temporary occlusion of the pulmonary circulation It has been suggested that such transient interruption of pulmonary blood flow might be responsible for some otherwise unexplained postoperative complications Therefore an experimental study has been made of the flow of blood within the lungs following occlusion and subsequent release of the left main pulmonary artery

METHOD

After consideration of a variety of methods for tracing this flow it was decided to label blood serum *in vivo* with radioiodinated serum albumin Radioiodinated I^{131} Serum Albumin (hereafter referred to as RISA) can be injected directly into the circulation with complete mixing occurring in minutes Its action thereafter is quite predictable small known amounts disappearing from the blood stream to appear in the thyroid kidney and extravascular protein pool [1] Serial hematocrit determinations permit the conversion of plasma levels to whole blood concentrations as needed The radioactivity of RISA is thus a convenient measure of both blood volumes and the mixing of labeled and unlabeled pools of blood

RISA was injected by turns into systemic or pulmonary vessels In different

* From the Mayo Clinic and Mayo Foundation Rochester Minn Supported in part by the Minnesota Heart Association

experiments this was done before during and after occlusion of the left main pulmonary artery At intervals following the injection sample amounts of exactly 1 ml were withdrawn via catheter from left and right pulmonary veins and systemic vessels All these samples were analyzed for radioactivity in a deep well scintillation counter The patterns of changing radioactivity of these samples and their progress to complete mixing (or equilibration) form the basis of this report

PULMONARY SYSTEMIC INTERCHANGE

Initially as a control RISA was injected into the left pulmonary artery of a normal dog (Fig 1) There was no occlusion of this artery Blood samples were withdrawn from the femoral artery and femoral vein As can be seen on the left this injected material appeared in the systemic circulation in seconds and equilibration was reached within 5 minutes The slight downward slope of the line beyond that point is produced by the disappearance of the radioactive material from the blood stream largely as a result of transfer to the extravascular protein pool

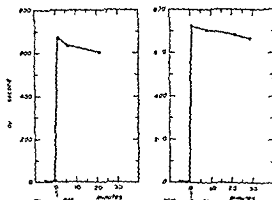


Fig 1 Patterns of equilibration in blood samples drawn from femoral artery and vein after injection of RISA into left pulmonary artery (left panel) and from pulmonary veins after injection of RISA into a peripheral systemic vessel (right panel)

In a separate experiment RISA was injected into a peripheral systemic vessel and blood samples were drawn from the pulmonary veins These samples were obtained via catheters passed into the left atrium and up the pulmonary veins in a retrograde manner The catheters (#190 polyethylene tubing) were passed well up into the veins to avoid left atrial contamination but were allowed to lie loosely in the veins in order not to impede blood flow The results of this experiment are shown on the right Injected material appeared rapidly in the lung and equilibration with systemic blood was quickly achieved

When the left main pulmonary artery was occluded temporarily by a tape a different effect was seen (Fig 2) In this experiment the RISA was injected into a superficial leg vessel after the occlusion It appeared at once throughout the systemic circulation as would be expected Samples drawn from the left pulmonary veins however showed a slow steady rise in radioactivity The rise continued over a period of approximately 2 hours until equilibration with systemic samples was reached Release of the left main pulmonary artery after this had occurred did not produce any further increase in radioactivity Thus the tagged blood from the body had appeared in the lung gradually replacing untagged blood trapped there by the occlusion in the absence of flow in the pulmonary arteries

In a third group of experiments (Fig 3) the left main pulmonary artery

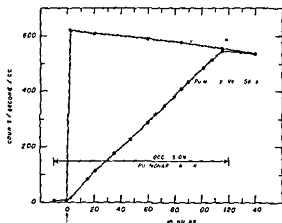


Fig 2 Patterns of equilibration in blood samples drawn from veins in the left cardiac and left diaphragmatic pulmonary lobes after injection of RISA into a superficial leg vessel and during temporary occlusion of left main pulmonary artery (Arrow at lower left marks injections of 0.1 cc RISA)

was occluded again, but the RISA was injected into this same artery just distal to the point of occlusion Only 0.1 ml was slowly injected to avoid forcing tracer material through the pulmonary tree Blood samples were withdrawn from the femoral artery and femoral vein As can be seen tracer appeared in the systemic blood and increased over a period of approximately 2 hours until equilibration was achieved That equilibration actually had occurred was indicated by three observations (1) the downturn of the curve of radioactivity signified that loss of RISA from the systemic blood was taking place at a faster rate than was the addition of further RISA from the occluded pulmonary artery (2) Equal degrees of radioactivity were found in samples drawn from the pulmonary veins draining the occluded lung *(This is not shown in the figure)* *(3) Calculation of the total blood* volume and comparison with the expected blood volume revealed distribution in the systemic blood of all the injected tracer material

In separate cases, this appearance and equilibration of injected tracer followed one of two differing 'typical' patterns both of which are shown Whichever was observed, the time required for complete washout of the tracer material from the occluded pulmonary artery was approximately 2 hours

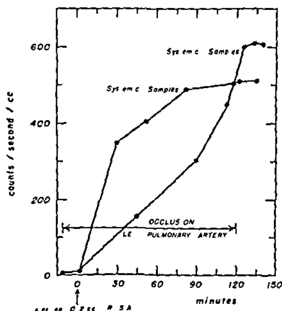


Fig 3 Two patterns of equilibration in blood samples drawn from femoral artery and vein after injection of RISA distal to temporary occlusion of left main pulmonary artery

ROLE OF BRONCHIAL CIRCULATION

At this point pulmonary-systemic interchange had been demonstrated adequately, but the mechanism of the action still was unexplained. Flow through the bronchial circulation seemed to be a likely possibility, but backward rinsing from the left atrium via the pulmonary veins or factitious withdrawal via the sampling catheters in the pulmonary veins could not be ruled out. Accordingly, an attempt was made to discover the factors responsible.

For this purpose the left main pulmonary artery was occluded again and, in addition, a tape was passed behind the pulmonary veins and around the remainder of the lung root so that the bronchial circulation could be interrupted while the pulmonary veins remained open to drain the lung (Fig. 4). The left pulmonary artery and the bronchial circulation to the left lung were occluded simultaneously and RISA was then injected into the left pulmonary artery distal to the site of occlusion. This tracer material did not leave the lung during a period of 90 minutes, despite the patency of the pulmonary veins. The series of blood samples drawn over that period showed no increase in radioactivity. The small counts shown are due to background radioactivity in the counting room.

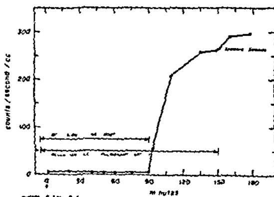


Fig 4 Pattern of equilibration in blood samples drawn from systemic vessels after injection of RISA into left pulmonary artery and during temporary occlusions of pulmonary and bronchial arterial flows to left lung

Upon restoration of the bronchial circulation, however, the tracer quickly appeared in the peripheral blood, and it continued to increase during the next hour Additional amounts also appeared with the release of the left pulmonary artery occlusion at the end of this time No catheters were placed in the pulmonary veins in this experiment The flow of blood to and from the lung in the absence of pulmonary artery flow seems dependent upon an intact bronchial circulation

As a corollary to this the effect of greatly increased bronchial flow was studied (Fig 5) Injections of RISA were given with the above-described techniques in dogs whose left pulmonary arteries had been ligated 3 years previously The bronchial circulation to these otherwise unaltered lungs had undergone tremendous increase in the interval The absence of pulmonary flow was verified anatomically prior to injection studies RISA was injected into the distal stump of this long severed pulmonary artery in the experiment shown here, and the tracer material appeared at once in the systemic circulation with complete mixing taking place in minutes In similarly prepared dogs RISA injected into peripheral systemic vessels appeared rapidly in pulmonary vein samples Increased bronchial flow, then, led to a decrease of equilibration time and rapid washout

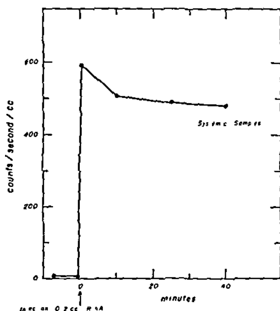


Fig 5 Pattern of equilibration in blood samples drawn from systemic vessels after injection of RISA distal to longstanding ligation of left pulmonary artery in the presence of resultant augmented bronchial flow to left lung

Finally, an attempt was made to demonstrate the relationship between the bronchial circulation and the pulmonary arterial circulation Connections between these two circulations in disease have been demonstrated by others, but the existence of bronchiopulmonary anastomoses in the normal dog had been questioned In this experiment RISA was injected into the peripheral blood stream after occlusion of the left main pulmonary artery One hour later, tiny samples were carefully drawn from the occluded pulmonary artery just distal to the occlusion and at some distance from the pulmonary capillary bed These blood samples showed increased radioactivity Because of the extremely small size of the pulmonary artery samples, exact comparison with systemic samples was not possible, but they did have a concentration of radioactivity approximately two thirds that of the peripheral samples Systemic blood, then, had appeared in the pulmonary artery *proximal to the capillary bed of the lung*

SUMMARY

It has been shown that continued flow of blood does take place in the lung following cessation of the pulmonary artery flow This occurs on both sides of the pulmonary capillary bed and is largely a function of the bronchial circulation

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RESPIRATION FOLLOWING LUNG DENERVATION *

L. Penfield Faber and T. J. Beattie, Jr

Recent experimenters performing homologous heart lung transplants in dogs have stated that the dog with totally denervated lungs is unable to resume spontaneous respirations and that the immediate cause of death was respiratory failure when mechanical respiration was discontinued [1,2] Previous work from this laboratory in which lungs were denervated for asthma and main stem bronchi were transplanted have never demonstrated the above described phenomenon of respiratory paralysis [3,4]

Injury to the phrenic nerve may be the reason for the failure to resume spontaneous respiration Complete sympathetic and parasympathetic denervation should alter only the respiratory rate and depth In the future, homologous lungs will be transplanted for certain diseases It is necessary to establish the feasibility of transplantation using autologous lungs Adult mongrel dogs were used for this study.

METHOD

To secure a completely denervated functioning lung, it was decided to do an autologous left lung transplant At a later date a right pneumonectomy would be performed Therefore, the dog could respire only with the denervated left lung The left side was chosen for the transplantation because of the fewer lobes present and the easier accessibility of the pulmonary artery.

Series 1. The dogs used weighed approximately 30 lbs and were anesthetized with intravenous pentobarbital sodium There were three dogs in this first series Following a complete hilar dissection, the left lung was removed from the chest cavity by severing the pulmonary artery, left main stem bronchus and a left atrial cuff The vascular system of the removed lung was then perfused with isotonic saline until all blood was washed free The appropriate anastomoses were then carried out using 5 0 silk for the atrium and pulmonary artery and 3 0 silk for the bronchus The chest was closed with an intercostal catheter attached to a waterseal bottle The time required to complete all anastomoses averaged about 3 hours

Series 2. In this group 10 dogs received a left autologous transplantation utilizing a different method The hilum was dissected free so that only bare bronchus, artery and veins remained During this dissection the phrenic nerve was carefully preserved The bronchus was then severed and immediately reanastomosed Heparin, 1 5 mg /kg, was injected into the left atrium Blood flow through the lung was interrupted and then the left atrial cuff was divided and reanastomosed The blood flow through the lung was reestablished for a few minutes The flow was again interrupted while the pulmonary artery was divided and reanastomosed All dogs received daily intramuscular penicillin injections postoperatively for one week

After periods varying from one to three weeks, the animals were re anesthetized and spirograms were made Immediately following this procedure a total right pneumonectomy was done This allowed the animal to respire only on the transplanted lung At the completion of the pneumonectomy, spirograms were repeated

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RESULTS

Series 1 All 3 animals died before the second stage of the procedure could be completed *The causes of death at autopsy were found to be* atelectasis pneumonia and thrombus formation in the pulmonary artery Microscopic sections of the transplanted lung parenchyma further revealed marked *pulmonary edema in 2 of the dogs Due to the failures* it was decided to revise the method of transplantation

Series 2 Three of the dogs in this group expired before the second stage *of the procedure could be completed Two of the deaths were* directly related to technical difficulties and the third death was a result of an overdosage of pentobarbital sodium while being anesthetized for the second stage

Seven dogs were alive and well at the time of the pneumonectomy and spirograms Serial chest x rays postoperatively revealed moderate amounts of pulmonary edema of the entire transplanted lung and in some dogs *atelectasis of the upper and middle lobes* The pulmonary edema ultimately cleared but the atelectasis persisted in some of the dogs despite bronchoscopy and assisted positive pressure breathing

Table 1 summarizes the results of all the spirograms Figure 1 illustrates typical spirograms from 2 of the dogs 12 and 21 days respectively after the left lung transplantation was done Dog 2 was breathing at a rate of 7 5 per *minute with an average tidal volume of 231 cc* and a minute volume of 1 73 liters Dog 3 was breathing at a rate of 30 per minute with an average tidal volume of 242 cc and a minute ventilation of 7 26 liters

Following the recording of the above tracings total right pneumonectomies were done After the chests were closed assisted respiration was maintained for approximately 15 to 30 minutes to allow the dogs to stabilize After discontinuance of the mechanical respirator *all of the dogs were noted to resume* respiration spontaneously Spirograms were then repeated Figure 2 illustrates the tracings of the same two dogs shown in Figure 1 after the right lung

Table 1

	SPIROGRAM BEFORE PNEUMONECTOMY		SPIROGRAM AFTER CONTRALATERAL PNEUMONECTOMY	
DOG	RESP RATE PER MINUTE	MINUTE VENTILATION LITERS/MINUTE	RESPIRATORY RATE PER MINUTE	MINUTE VENTILATION LITERS/MINUTE
1	30	5 18	10	1 90
2	[illegible]	1 73	17 5	3 [illegible]9
3	30	7 26	11 2	2 34
4	8	1.54	17	0 86
[illegible]	[illegible]	3 [illegible]	20	2 32
6	11 [illegible]	4 21	10	[illegible]
7	16	3 20	12	2 14
Average	18 3	3 84	13 9	2 21

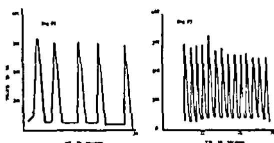


Fig 1 Spirograms made before the right contralateral pneumonectomy Dog 2 is 12 days posttransplantation and dog 3 is 21 days

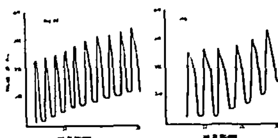


Fig 2 Spirograms made following right contralateral pneumonectomy on the same 2 dogs shown in Figure 1 Both dogs have resumed respirations spontaneously

has been removed Dog 2 increased his respiratory rate to 17 5 per minute The average tidal volume was reduced to 205 cc but the minute ventilation was 3 59 liters Dog 3 reduced his rate to 11 per minute but decreased his tidal volume to 209 cc, and his minute ventilation to 2 31 liters

The survival time after pneumonectomy varied from 2 to 12 hours with an average of 5 5 hours All 7 dogs expired from air hunger and anoxia Autopsy revealed atelectasis and pleural fibrosis which limited expansion of the pulmonary parenchyma and appeared to be the immediate cause of death All anastomotic sites were patent and free of thromboses In all instances the atelectasis involved mainly the upper and middle lobes The lower lobe appeared to be functioning well Microscopic examination of the transplanted parenchyma revealed no evidence of change in the capillary membrane or alveolar wall

We feel that it is impossible for any of the nerve fibers to have regenerated as they were all unmyelinated and their approximating ends were radically disrupted With the phrenic nerve preserved the diaphragm was able to function adequately and spontaneous respiration was resumed by the autologous transplanted left lung

SUMMARY AND CONCLUSIONS

1 Thirteen dogs received left autologous lung transplantations

2 Seven survived the initial procedure and a contralateral pneumonectomy was done

3 All 7 of the dogs resumed spontaneous respiration which was confirmed by spirogram

4 Causes of death were attributed to difficulty in expansion of pulmonary parenchyma

5 The denervated lung is able to resume spontaneous respiration if the phrenic nerve is preserved

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Table 2 Effects of Breathing 100% Oxygen for 7 Minutes

PATIENT	ROOM AIR		100% OXYGEN	
	CO_2 CON (VOLS %)	O_2 SAT (%)	CO_2 CON (VOLS %)	O_2 SAT (%)
		PICKWICKIAN SYNDROME		
H H	48 95	82 06	49 77	89 15
J F	3 38	85 44	[illegible]7 0[illegible]	91 57
I M D	59 00	80 66	59 98	80 84
W A McI	68 72	86 9[illegible]	61 41	91 [illegible]7
E J	49 17	88 11	48 60	95 96
		SIMPLE OBESITY		
A McG	42 51	92 65	44 72	93 57
P S	45 71	91 77	43 47	96 99
E M F	47 55	92 32	45 54	91 06
C T	49 90	92 75	51 68	89 35

DISCUSSION

There was little difference in the findings in the two groups other than the presence of oxygen unsaturation and carbon dioxide retention The possibility that this might represent abnormal circulatory patterns was considered and this was evaluated by testing their ability to achieve complete arterial oxygen saturation by the inhalation of 100% oxygen for 7 minutes Nine patients were studied including 5 of the Pickwickian group (Table 2) None of the Pickwickian group achieved complete saturation and in 3 of the five there was a rise in carbon dioxide content In the 4 patients without obvious oxygen unsaturation only one patient achieved the anticipated 97% saturation and in 2 the carbon dioxide content rose These studies suggest that if circulatory abnormalities in the form of unusually large physiologic shunts do occur they are present in both those with and without obvious oxygen unsaturation There is also a possibility of an abnormal respiratory center response to carbon dioxide but this point has not been investigated

Four patients were evaluated as to the effect of altering their position on blood gas and ventilation values (Table 3) In 3 of the 4 the resting minute volume increased in the seated position with an increase in alveolar ventilation Blood gas values were improved in 2 patients unchanged in one and made worse in one These responses to changes in position are insufficient to explain the differences in alveolar ventilation reported in various studies [1-4] It would also indicate that no predictable amount of improvement could be expected from placing the patients in an upright position though individual patients may find breathing easier in one position than in another

Three of the patients (R H E M D & L M D) subsequently had surgery involving a general anesthetic Death occurred postoperatively in the only patient of the 3 who had oxygen unsaturation (L M D) One other extremely obese patient (400 lbs) not included in the series because of incomplete preoperative studies had a minimal oxygen unsaturation preoperatively (90 5%)

Table 3 Effect of Position

	Recumbent			Seated		
Patient	pCO_2 (mm Hg)	O_2 sat (%)	Alv vent (L/min)	pCO_2 (mm Hg)	O_2 sat (%)	Alv vent (L/min)
H H †	46	82 06	5.50	43	81.29	6 81
M B	50	90 27	4 02	48	96 71	5 11
E M O	37	91 31	5 13	35	97.26	5.30
L J	36	90 96	4 31	37	87.39	5.32

† "Pickwickian Syndrome

and died immediately postoperatively, presumably of cardiac arrest The surgical procedure was an emergency laparotomy for an incarcerated and strangulated ventral hernia of 3 days duration Here it seemed that inadequate respiratory exchange caused primarily by the massive weight of her chest and abdomen was a factor in her death While these problems deserve further investigation blood gas studies appear to offer some help in evaluating such patients

These studies would suggest that obesity in both the patients with oxygen unsaturation and those without oxygen unsaturation constitutes a complex overload on the cardiorespiratory system The increased work of respiration referred to by other authors[1-5] probably is not the only factor An increased oxygen supply is necessary with increasing weight and while the lung with hyperventilation can achieve far greater oxygen uptake than any of these patients demonstrated,[6] the efficiency of the mechanism is markedly reduced Earlier studies have shown an increasing cardiac output with an increase in weight[7] Certainly a limit could be reached beyond which either or both the lung and heart could no longer supply the demands of the body Hence the term cardiopulmonary failure as suggested by Estes[3] and Lillington[5] seems most appropriate

SUMMARY

Pulmonary function studies have been performed in patients displaying marked obesity The patients were divided into a group representing the Pickwickian Syndrome and a group displaying simple obesity, but with the exception of oxygen saturation and carbon dioxide retention there was little difference in the two groups Simple ventilatory studies proved inadequate for denoting poor risk patients It would seem that blood gas studies should be a part of the evaluation of the obese patient for surgery

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HISTOLOGIC STUDIES OF THE LUNG DURING RECOVERY FROM ATELECTASIS *

J. RAYMOND HINSHAW AND GEORGE L. EMERSON

It has been demonstrated clinically that restoration of bronchial continuity months or even years after traumatic bronchial rupture will result in an aerating, apparently functioning lung. Experimentally, it has been shown that a chronically atelectatic lung will regain function following reexpansion.[2] Information on the effects of obstructive atelectasis on the tissues of the lung is, however, limited. The histology of pulmonary parenchyma after long standing atelectasis has been variously reported, but striking pathologic changes have seldom been noted. It is generally agreed that the bronchial lumen distal to an obstruction is increased in diameter, and some observers hold that this is truly experimental bronchiectasis. Since interpretation of pulmonary architecture, and even of cellular detail, in the atelectatic lung is not easy, artificial inflation of the experimentally atelectatic lung has been employed immediately after pneumonectomy.[1-3] Although the ability of such a lung to expand can be demonstrated in this manner, the difficulty with which expansion is achieved does not necessarily indicate permanent alterations in the pulmonary tissue. Nor does artificial inflation with considerable pressure seem an ideal way to obtain tissue for histologic interpretation. One study has suggested that obstructive atelectasis followed by reexpansion of the collapsed lung results in no discernible change in the pulmonary parenchyma.[4] In order to learn how rapidly, how completely, and by what means the chronically atelectatic lung is restored toward normal, the following experiments were performed.

METHOD

The experimental animals were 13 adult mongrel dogs. In 4, the right upper lobe bronchus was transected and each end was closed with interrupted 5-0 silk sutures. In 9, the right upper lobe bronchus was occluded with a heavy silk ligature. Occlusion of the bronchus was complete in 6 animals and incomplete in 3. In one of the latter 3, complete occlusion was achieved at a second operation, but in the other 2 lobectomy was performed to obtain tissues for the study of the effects of incomplete occlusion.

At periods varying from 5 weeks to 10 months after the original operation, bronchial continuity was reestablished by excision of the stenosed segment and anastomosis of the cut ends with interrupted 5-0 silk sutures. The right upper and middle or lower lobes were biopsied both at the time of bronchial interruption and after bronchial anastomosis. From 1 to 3 additional biopsies were performed at intervals varying from one week to one and one half years after restoration of the airway to the lobe. At other times, bronchoscopy and x-ray films permitted evaluation of the anastomoses and the aeration of the lobes. The final procedure was a right pneumonectomy performed to obtain specimens of the anastomosis, the bronchi and the lung parenchyma. Sections were stained with hematoxylin and eosin and with Verhoeff's stain for elastic tissue.

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RESULTS

Gross Appearance The small size liverish appearance and rubbery consistency of the atelectatic lobe is too well known to require detailed description This was found in 9 of the 13 animals In 3 the ligature had narrowed the bronchus to about one fourth its original diameter but the lobes looked and felt normal In one of these the incompletely occluded bronchus was retied with resulting complete atelectasis One animal became sick nearly 3 months after the original operation At exploration the right upper lobe was greatly enlarged and had the consistency of liver but was crepitant in small areas Purulent material was aspirated from the bronchus which was occluded completely near the trachea and the airway was reestablished after resection of the stenosed portion Apparently an incompletely ligated bronchus had been completely obstructed by inflammatory reaction following infection

When the stenotic portion of the bronchus was resected much viscid gelatinous mucus oozed from the distal end After aspiration of this material dilatation of the bronchi was evident The degree of dilatation seemed no more at the end of 10 months of occlusion than after 5 weeks of atelectasis

When the airway was reestablished expansion of the right upper lobe was accomplished with relative ease Expansion to normal size occurred rapidly but the tissue always appeared paler than the other lobes and the consistency retained some of the rubbery character of the atelectatic lobe

If the first biopsy was performed one week after bronchial anastomosis a slightly pale slightly rubbery lobe with a little lag during the inspiratory phase was found Exploration one or two weeks later revealed well expanded lobes of normal color and nearly normal consistency If the lung was not disturbed for one month after restoration of the airway no gross abnormality was noted

Microscopic Appearance The histologic changes described below as occurring following obstructive atelectasis are not prominent They should really be considered the exception rather than the rule but they are presented as differences between the experimental and the control lobes They represent what happens to a minority of the cells or tissues involved and it is fair to state that reexpansion of the chronically atelectatic lobe results in nearly normal lung tissue

Bronchi The segmental bronchi though noticeably dilated by mucus during the term of bronchial occlusion retain their normal mucosa and return to normal size within at least one month after restoration of the airway In only one specimen could any permanent effect on the smaller bronchioles be questioned For one or two weeks after restoration of the airway occasional mucus plugs and leucocytes were found in the bronchioles The walls of both the bronchi and the bronchioles contained normal muscle fibers and normal elastic tissue both during the atelectatic period and following reexpansion

Blood vessels Very rarely thrombosed small vessels are found both centrally and peripherally They represent such a minority of the vascular supply of the lung t[illegible] degree Some of the smalle[illegible] [illegible]ding atelectasis but the el[illegible] tissue remains normal The bizarre vascular formations which have been reported [1] are not seen

Alveoli and alveolar septa Disruption of some alveolar walls occurs after positive pressure reexpansion Although this was evident immediately after reexpansion later biopsies generally showed a normal architecture

Within the air spaces numerous rounded cells with small dark nuclei and pale vacuolated (foamy appearing) cytoplasm were found in the atelectatic lung Immediately after reexpansion similar cells could be identified in the septa as well Within a week or two after reexpansion they were no longer prominent

The elastic tissue of the alveolar septa underwent no noticeable change as a result of atelectasis

Connective tissue An increased amount of connective tissue was found around some blood vessels but if infection was absent prominent fibrosis did not occur

Pleura The thickening of the visceral pleura was no more than would be expected from the repeated thoracotomies and was little more than that found on the other lobes of the right lung

CONCLUSION

When infection is not superimposed chronic obstructive atelectasis produces few changes in pulmonary tissues The changes which do occur are subtle some are easily reversed within weeks of reexpansion of the lung and none of them should significantly alter pulmonary function permanently

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ISOLATION OF THE ELASTIC TISSUE NETWORK OF THE LUNG *

ROBERT W CARTON BEVERLY TEWS
C BRUCE TAYLOR AND GEORGE M HASS

Elastic tissue is widely believed to be a principal agent in the elastic recoil of the lung and alterations in the elastic tissue network are looked for when this elastic recoil is impaired Although the effects of altered pulmonary elasticity on gas and blood flow have been studied in great detail the structural background of these changes is not well understood A method by which the pulmonary elastic tissue can be studied in three dimensions free from surrounding structures should be helpful in elucidating its form and physical properties

* From the Departments of Medicine Pathology and Surgery of the Presbyterian St Lukes Hospital and The University of Illinois College of Medicine Chicago Supported in part by grants (H 1630) from the National Heart Institute of the National Institutes of Health and the Otho S A Sprague Memorial Institute

The arterial wall, similarly, is a mixed tissue with important elastic qualities Arterial elastic tissue has been isolated [1] by the use of warm formic acid, and it has seemed reasonable to apply an analagous method to the study of the lung

METHOD

Blocks of fresh lung about 2 cm in each dimension were taken from surgical and autopsy specimens and treated by immersion for 72 hours in an excess of 88% formic acid at 15°C The residual network could then be seen under the dissecting microscope to have a structure resembling that previously described for the elastic tissue network of the lung [2] This material was then neutralized in running tap water, stained with aqueous basic fuchsin, frozen in tap water, dried by the freeze dry technique, and embedded in ethyl or butyl methacrylate.

We feel that this formic acid resistant tissue represents nearly pure elastica because, (a) it accepts the Weigert elastic stain readily, (b) it has an appearance under the electron microscope compatible with that of elastic fibers,[3] and (c) it retains the architecture of the elastic network of the lungs as seen in thin sections

RESULTS

This tissue as isolated forms a skeleton of the primary lobule, and the route of the airways can be followed from the alveoli through the alveolar ducts to the smaller bronchioles Certain features of the normal preparation are noteworthy The tissue as a whole forms a dense meshwork (Fig 1), the majority of the fibers being located in the position formerly occupied by the alveolar walls The alveolar ducts are represented by connecting "hoops" of fibers, and these, placed one after another, outline an airway The elastic tissue of the blood vessel walls is preserved, hence the path of the vessels can be traced into the framework of the lung

Corrosion preparations by this method were made of lungs from two persons suffering from severe pulmonary emphysema In both instances the air spaces were highly irregular in size and distribution. The elastic fibers were scarce in comparison with those of normal lungs, and many appeared thick and stained darkly

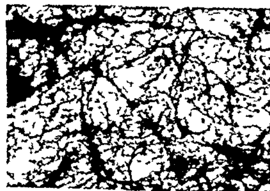


Fig 1 Elastic tissue network from the normal human lung Magnification 60X

CONCLUSION

A method is described for the isolation of the elastic tissue network of the human lung By the use of this technique, the elastic tissue can be studied in three dimensions in normal lungs and in those in which elastic recoil is impaired

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ELECTRICAL ANESTHESIA SOME METABOLIC OBSERVATIONS AND COMPARISONS *

CURTIS D MCNEIL, M D TURNER, AND JAMES D HARDY

High frequency currents applied to the head of an animal produce instantaneous surgical anesthesia [1] This effect can be completely and immediately reversed by stopping the current It appears that electrical anesthesia could be controlled more easily than any other anesthetic agent However, a number of undesirable side effects have been observed, some of which have been partially overcome [1] The adrenal medullary and adrenal cortical response to electrical anesthesia is compared here with two of the more familiar general anesthetic agents Adrenal medullary activity was followed by measuring the circulating levels of epinephrine and norepinephrine Adrenal cortical response was followed by serial determinations of blood levels of the 17, 21 dihydroxycorticoids

METHOD

Twenty eight dogs were divided into 4 groups of 7 animals Three groups were subjected to different general anesthetic agents and a standard midline celiotomy of 30 minute duration was performed Anesthesia was induced in one group by the use of an electrical current Each dog in this group received 5 to 10 units of d tubocurarine before induction of anesthesia to facilitate endotracheal intubation The dose of tubocurarine given was subparalytic, all animals were capable of respiration after the drug was administered Characteristics of the electrical current used were similar to those used previously by Knutson, *et al* [1] In this case a 700 cycles per second current was applied, operating at about 15 volts and 50 milliamperes

The second group was anesthetized with pentobarbital and the third group by ether The remaining group of animals received no general anesthetic, instead procaine was injected locally at the site of the incision

Thirty milliliter control blood samples were obtained from a femoral vein of all animals before initiation of the procedures outlined above Additional blood samples were obtained midway through the operative procedure, at the end of the procedure, and 1, 2, and 2½ hours after completion of surgery The blood plasma was analyzed for 17 21 dihydroxycorticoids according to the method of Nelson and Samuels [2] Plasma concentrations of epinephrine

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* From the University of Mississippi Medical Center Jackson Supported by Army Contract Number DA 49 007 MD 627

and norepinephrine were determined by the method of Weil-Malherbe and Bone,[3] as described by Mangan and Mason.[4] However, we were able to obtain better results by the use of Richardson's filters.[5]

Since the analytical procedures required large amounts of blood the animals were transfused with an equal volume of blood after each sample was drawn.

RESULTS

Tables 1, 2 and 3 give the mean circulating plasma values of 17, 21-dihydroxycorticoids, epinephrine, and norepinephrine obtained from the four groups of animals studied. The group that received procaine locally was considered to be a control group from which the effect of the surgical procedure alone could be evaluated. Any deviation from this group observed in the groups receiving general anesthetics was assumed to be due to the anesthetic agent used.

Animals that received only procaine exhibited a small but consistent rise in hydroxycorticoids, whereas animals that received pentobarbital showed a slight decrease or no change. Hydroxycorticoid levels in the group receiving ether were about the same as the group receiving procaine. The group receiving electrical anesthesia exhibited a marked rise in hydroxycorticoids reaching a peak one hour after the current had been stopped.

Table 1. Mean Plasma Levels of Free 17-Hydroxycorticoids of Dogs Subjected to Different Anesthetics Plus a Standard Celiotomy (Micrograms/100 ml. Plasma)

TYPE ANESTHETIC	NUMBER OF ANIMALS	CONTROL	MIDWAY SURGERY (15 MIN.)	END OF SURGERY (30 MIN.)	1 HOUR AFTER	2 HOURS AFTER	2.5 HOURS AFTER
Procaine	7		15.1	13.3	14.4	12.7	11.8
Electrical anesthesia	7	10.4	16.2	17.9	20.0	15.6	11.7
Pentobarbital	7		12.3	12.8	11.3	11.7	13.5
Ether	7		11.6	15.8	14.3	13.8	10.7

Table 2. Mean Plasma Levels of Epinephrine in Dogs Subjected to Different Anesthetics Plus a Standard Celiotomy (Micrograms/Liter Plasma)

TYPE ANESTHETIC	NUMBER OF ANIMALS	CONTROL	MIDWAY SURGERY (15 MIN.)	END OF SURGERY (30 MIN.)	1 HOUR AFTER	2 HOURS AFTER	2.5 HOURS AFTER
Procaine	7		0.58	0.59	0.57	0.46	0.48
Electrical anesthetic	7	0.28	0.58	1.07	1.15	0.57	0.47
Pentobarbital	7		0.04	0.13	0.23	0.16	0.21
Ether	7		0.46	0.45	0.44	0.54	0.54

Table 3 Mean Plasma Levels of Norepinephrine in Dogs Subjected to Different Anesthetics Plus a Standard Celiotomy (Micrograms/Liter Plasma)

TYPE ANESTHETIC	NUMBER OF ANIMALS	CONTROL	MIDWAY SURGERY (15 MIN)	END OF SURGERY (30 MIN)	1 HOUR AFTER	2 HOURS AFTER	2.5 HOURS AFTER
Procaine	7		3.25	3.15	3.36	3.58	3.22
Electrical anesthetic	7		3.12	3.34	3.76	4.18	3.33
Pentobarbital	7	2.83	2.73	2.45	2.37	2.80	3.38
Ether	7		2.50	3.51	2.46	2.50	2.52

A striking rise in plasma epinephrine levels was observed in animals receiving the electrical anesthetic (Table 2). The epinephrine levels reached a peak at one hour after the current was stopped. After this time the epinephrine concentrations rapidly declined to control values.

The procaine group exhibited a considerable rise in epinephrine during the surgical procedures. Since little change occurred in epinephrine levels after pentobarbital anesthesia, it was assumed that this agent inhibited the rise of circulating epinephrine. The increase in epinephrine concentration in the ether group was somewhat less than the increase observed in the procaine group.

No marked alterations in norepinephrine levels occurred in any of the four groups studied, but the direction of the changes observed was the same as those found for epinephrine (Table 3). A small but consistent rise was observed in the animals that received the electrical anesthetic, whereas pentobarbital appeared to prevent any increase in norepinephrine levels.

DISCUSSION

Electrical anesthesia causes excitation of the sympathicoadrenal system as shown by the increased blood concentration of epinephrine. Elevated hydroxycorticoid levels were also exhibited in these animals, indicating a rise in adrenal cortical activity during electrical anesthesia. In fact, the response to high frequency currents was considerably greater than that produced by the minor surgical procedure itself. On the other hand, pentobarbital shows a definite inhibitory effect on both adrenal medullary and adrenal cortical function. Ether appears to produce an effect intermediate between the two agents mentioned above.

Electrical anesthesia as produced in these studies appeared to subject the animal to severe stress. However, this agent permits the adrenal medullary and adrenal cortical systems to respond freely. Pentobarbital, on the other hand, seems to have a depressive effect on the systems studied. It would appear that the more desirable anesthetic agent is one that permits an uninhibited response of the body to surgical stress. Although electrical anesthesia permits such a response, it is far more traumatic than the stress of surgery. The intermediate response observed in the animals anesthetized with ether appears to be closer to the response desired.

CONCLUSIONS

1 Electrical anesthesia produced an increased elaboration of hydroxycorticoids, epinephrine, and norepinephrine considerably above that observed in animals subjected to operative stress alone

2 Ether anesthesia had little effect on the secretion of epinephrine, norepinephrine, and hydroxycorticoids

3 Pentobarbital produced a depression of both adrenal cortical and adrenal medullary activity in animals subjected to surgical stress

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FAT EMBOLISM, ETHER CONVULSIONS AND NEUROLOGIC DEFICITS *

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Convulsions in previously normal individuals are rare but disastrous complications of ether anesthesia Reproducible phenomena of this type accurately reflecting the clinical picture have been consistently produced in animals by combining induced hyperthermia and diethyl ether anesthesia [1] These studies have demonstrated (1) the value of the electroencephalogram in early detection of abnormal cortical activity during anesthesia, (2) that among other anesthetic agents similarly studied (divinyl ether, pentothal, pentothal plus ether, cyclopropane, chloroform, nitrous oxide and ethylene) only divinyl ether produced cortical arrhythmias but unlike diethyl ether, no central nervous system damage was observed, and (3) that the mechanism of the cytotoxic changes in the brain brought about by the combination of hyperthermia and diethyl either anesthesia was not clear

This report deals with studies undertaken in order to develop further some of the specific metabolic changes which occur with ether convulsions

METHOD

After withholding food for 24 hours 2 groups of 10 healthy dogs each were anesthetized with open drop ether (diethyl) After endotracheal intubation, ether was administered with 100% oxygen through a closed gas system in

* From the S R Light Laboratory for Surgical Research and the Departments of Anatomy and Surgery Vanderbilt University School of Medicine Nashville Supported by U S P H Grant B 1191(R1)

which a soda lime carbon dioxide absorber was incorporated. In Group A body temperatures (measured rectally) were elevated to 105 to 107°F using a heated rubber blanket. No preanesthetic medication was employed. Group B dogs were made hyperthermic in the same manner during ether anesthesia to levels ranging from 110° to 112°F. Sodium heparin (10 mg/kg) was administered intravenously to Group B dogs prior to anesthetization. In all animals continuous unipolar and bipolar EEG tracings were obtained. Femoral blood pressures and transthoracic ECGs were recorded also. Blood samples were collected from the femoral artery prior to and one hour after anesthetization, and finally during the occurrence of any EEG abnormality. If none occurred, a blood sample was obtained at the end of the experiment. Total serum lipid, cholesterol and phospholipid determinations were made. All surviving animals were sacrificed between 2 and 14 days following the procedure. Brain, lung, and kidney tissue from all animals was fixed in 10% formalin for 24 hours. Frozen sections of these tissues were then stained with Sudan IV. In those animals demonstrating the most marked neurologic deficits brain tissue was embedded in paraffin, stained with hematoxylin and eosin, and sections from this material were examined microscopically for pathologic changes.

RESULTS

All dogs of Group A developed EEG recorded seizures at temperatures ranging from 102 to 106.5°F. After recovery from anesthesia each animal besides loss of balance demonstrated behavioral changes characterized by high pitched continuous yapping and marked belligerency. Seven of this group regained normal ambulation and became docile during the succeeding 24 hours. Another animal remained profoundly affected neurologically with disturbed ambulation and balance, atonia and tremor until sacrificed 2 weeks later. Two other dogs expired within 24 hours following termination of the experiment without having regained any semblance of normal central nervous system function.

Serum lipid determinations are illustrated in Table 1. A noteworthy decrease in phospholipids of 30% or more occurred in those animals which demonstrated severe neurologic defects and, as will be noted later, significant amounts of cerebral fat emboli were observed. No relationship appeared to exist between the EEG recorded seizures or neurologic defects and total serum lipid levels.

The 8 surviving animals were sacrificed from 24 hours to 2 weeks after the experiment. Sudan IV stained sections demonstrated pulmonary fat emboli in all 10 animals. The greatest amounts were found in the 2 dogs which died as a result of the experiment. None of the animals were found to have stained fat within kidney glomeruli. The two animals which died as a result of the experiment and that one which developed profound and persistent neurologic damage had large quantities of embolic fat located within the lumen of cerebral and cerebellar capillaries. The latter animal also had large amounts of fat located within cells lining the capillaries. The concentrations of intracellular capillary fat in this dog which was allowed to survive for 2 weeks suggests that plugged vessels may have been opened by phagocytic action of these cells. Similar fat laden cells lining cerebral capillaries in the temporarily affected dogs were observed occasionally. These possibly are clasmatocytes which normally may be found filled with lipid material.

Table 1 Responses of Group A Dogs to Ether and Hyperthermia (105 to 107°F)

DOG	TOTAL LIPID MG %	CHOLESTEROL MG %	PHOSPHOLIPID MG %	FAT EMBOLI OF BRAIN	NEUROLOGIC DEFICIT	SEIZURES
639 Control / During Seizure	↓ 439 / 327 25%	↓ 152 / 110 21%	↓ 15.5 / 11.5 22%	+	Neg	++++
619	↓ 586 / 473 19%	↑ 169 / 176 9%	↓ 14.0 / 13.0 7%	Neg	Neg	++++
679	↑ 473 / 481	↑ 136 / 151	↑ 11.6 / 12.7	Neg	Neg	++++
671	↑ 391 / 416	↔ 107 / 107	↑ 8.8 / 9.2	Neg	Neg	++++
612	↓ 405 / 235 41%	↓ 111 / 133	↓ 10.0 / 7.0 30%	++++	Positive DIED	++++
627	↓ 173 / 152 15%	↑ 110 / 181	↓ 12.0 / 10.0 18%	Neg	Neg	++++
711	↓ 473 / 150 12%	↓ 165 / 151	↓ 11.8 / 10.0 32%	++++	Positive DIED	++++
744	↓ 383 / 338 11%	↓ 70 / 65 7%	↓ 7.5 / 6.1 18%	Neg	Neg	++++
797	↑ 485 / 577	↓ 120 / 100	↑ 11.0 / 12.1	Neg	Neg	++++
955	↑ 315 / 326	↓ 110 / 103	↑ 5.8 / 3.8 31%	++++	Positive	++++

Values represent determinations prior to the experiment and during seizure discharge

Table 2 Responses of Group B Dogs to Ether and Hyperthermia (110 to 112°F)

DOG	TOTAL LIPID MG %	CHOLESTEROL MG %	PHOSPHOLIPID MG %	FAT EMBOLI OF BRAIN	NEUROLOGIC DEFICIT	SEIZURES
745 Control During Seizure	↑ 788 812	↑ 252 285	↑ 19 8 22 0	Neg	Neg	–
766	↓ 394 372	↓ 110 100	↓ 7 5 6 0 20%	+	Neg	–
773	↑ 158 180	↑ 52 56	↑ 3 7 4 5	Neg	Neg	–
776	↔ 440	↑ 125 130	↑ 11 8 12 9	Neg	Neg	–
791	↓ 754 597	↓ 225 190	↓ 18 7 16 1 17%	Neg	Neg	–
807	–	–	–	Neg	Neg	–
797	↓ 281 259	↑ 72 82	↑ 5 0 6 5	Neg	Neg	–
744	↑ 406 440	↓ 146 138	↑ 12 1 13 7	Neg	Neg	–
989	↓ 417 293	↓ 152 119	↓ 6 4 3 4 47%	+++	Positive	++++
990	↓ 383 293	↓ 162 130	↓ 5 2 4 1 21%	+	Neg	–

Values represent determinations prior to and at the termination of the experiment

Previous work[2] has identified the cerebellar Purkinje cells as the most severely affected of the central nervous system when neurologically defective animals have been produced under these experimental circumstances. In the one animal in Group A which sustained severe and prolonged neurologic damage the absence of cerebellar Purkinje cells was again remarkably striking.

Nine of 10 animals in Group B subjected to hyperthermia (110 to 112°F.) and ether anesthesia *after heperinization* failed to develop EEG seizure patterns. Postexperimental recovery was prompt and unattended by behavioral or motor changes customarily seen in Group A dogs during this period. None of these 9 animals developed neurologic disturbances. Burns of the body occurred frequently since blanket temperatures ranged around 150°F. during the 2 to 3 hours of the experiment. One animal (989) developed an EEG recorded seizure at a temperature of 111°F. after 2 hours of exposure to heat and ether. During the immediate recovery period, and later, this animal demonstrated central nervous system changes similar to those observed in Group A dogs. All Group B dogs survived the experiment and the immediate postexperimental period. No attempt was made to reverse the heparin effect. Table 2 records the lipid responses. Dog 989 developed marked decreases in all fractions, with a phospholipid decrease of 47%.

Group B dogs were sacrificed from 24 hours to 6 days following the experiments. No pulmonary or renal fat emboli were found. Three animals demonstrated occasional fat deposits located in cells making up the wall of cerebral and cerebellar capillaries. This may well represent normal lipid distribution within the vessel walls. However, dog 989 had considerable amounts of fat located within the lumen of cerebral capillaries. Permanent sections from this animal revealed some decrease in the number of cerebellar Purkinje cells.

COMMENT

The present experiments have demonstrated an apparent correlation between the central nervous system damage induced by hyperthermia and ether and fat emboli. In 1927, Lehman and Moore[3] demonstrated that ether added *in vitro* to prepared fat emulsions or to human plasma precipitated homogenized fat in all instances. Animals killed by inhaled or intravenously administered ether were also found to have varying amounts of coalesced fat present in the lungs.

Ether in smaller amounts but with hyperthermia added has, under the circumstances of this experiment, produced similar results. A most significant finding in the present study lies in the dramatic effect of heparin in reducing the CNS reactions to ether and hyperthermia. Without excluding the "clearing" effect of heparin as a possible explanation the mechanism of its action might lie in this agent's capacity to prevent sludging of blood during hyperthermia.[4] Anoxia produced by sludging of blood added to intermittent occlusion of capillaries by fat might readily produce the central nervous system pathology encountered in experimental and clinical ether convulsions.

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A STUDY OF RESPIRATORY ARREST FOLLOWING THE ADMINISTRATION OF NEOMYCIN *

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One of the most valuable contributions to modern surgery has been the advent of antibiotic therapy but at the same time, there has been a proportional increase in the associated complications of such therapy At the present time more than 3000 antibiotic agents have been investigated, but less than 2 dozen are in commercial production One of the most difficult demands on the performance of an antibiotic is that the material possess a minimum of toxicity within the dose range needed for effective use Occasionally a specific toxic manifestation will not become obvious until the drug has been in common use for some time

In 1919 Waksman and Lechevalier,[1] working with soil microorganisms derived an antibiotic which they called neomycin This drug exhibits a broad and effective spectrum of antibacterial activity, but because of the nephrotoxicity and ototoxicity associated with its parenteral administration, it is used principally as an intestinal antiseptic and as a topical antibiotic

In 1956 a third striking toxicity of neomycin therapy was emphasized by Pridgen [2] who reported 4 cases cases of respiratory arrest which he attributed to the use of intraperitoneal neomycin At the present time a total of 11 cases of neomycin induced apnea have been reported in the literature, and in 5 of these, the patients have died In each instance, neomycin had been used to combat peritoneal contamination and was considered responsible for the respiratory arrest Pittinger and Long [3] have suggested that the underlying cause of the apnea referred to by these clinical reports was probably a curare like activity of neomycin producing a blockade of the neuromuscular junction with subsequent paralysis They also suggest [4] that this effect is potentiated when ether, which has a similar property, is the anesthetic agent

The present study was undertaken to determine whether neomycin effects a central respiratory depression as well as neuromuscular blockade and whether peritoneal trauma alters absorption of intraperitoneally administered drugs sufficiently to be a significant factor in the production of lethal serum levels Experiments to confirm the reports of neostigmine antagonism to neomycin induced respiratory arrest were also carried out The authors do not present the following data as a completed study, but rather as a progress report

METHOD

Adult mongrel dogs weighing 7 to 10 kilograms were anesthetized with 70 mg /kg chloralose prepared as a 5% solution in 10% urethane (Chloralose does not depress respiration except late in the course of anesthesia and leaves the cardiovascular system relatively unaffected Urethane was used primarily to enhance chloralose solubility but it also produces a narcosis in animals without significant depression of respiration circulation or spinal reflexes) The animals with contaminated peritonea were prepared by percutaneous injection of a fecal saline mixture *into the peritoneal cavity* Test

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injections into these animals were made after 8 to 12 hours Electrocardiogram and blood pressure were recorded on an eight channel Grass electroencephalograph Respiratory rate and volume were measured by means of a Metro Canine Respirometer Test doses of neomycin sulfate in saline solution were injected intravenously intraperitoneally and intrathecally Samples of venous blood and cerebrospinal fluid were taken at intervals of 5 10 20 40 and 80 minutes following a test injection or at time of death Each animal served as its own control Plasma and cerebrospinal fluid neomycin levels were determined by turbidometric bioassay utilizing *E coli* and *B subtilis* as test organisms The method consisted of comparing two fold dilutions in tryptose phosphate broth of a known amount of neomycin dissolved in control serum (CSF etc) obtained from the subject with similar preparation of the test fluid in which the neomycin concentration was to be determined Both sets of tubes were innoculated with a test organism of known age volume and dilution The concentration of neomycin in the test fluid was calculated by multiplying the concentration of neomycin that would inhibit the test organism by the dilution of the test fluid that gave the same inhibition

RESULTS

In order to determine whether neomycin affects central respiratory depression as well as peripheral neuromuscular blockade a three phase investigation was begun Cerebrospinal fluid was examined for neomycin buildup after developing high neomycin levels in the peripheral blood neomycin was injected intrathecally and the phrenic nerve was monitored for altered frequency of discharge during the apnea induced by a large intravenous dose of neomycin (1) Each of 4 dogs was injected intravenously with 175 mg /kg neomycin and developed immediate apnea The average plasma level attained at this dose was 300 μg /ml while CSF samples obtained during the period of apnea assayed consistently less than 0 6°5 μg /ml (2) Two dogs received cisternal injections of neomycin in amounts sufficient to develop CSF concentrations up to 500 μg /ml This procedure caused occasional emesis but no depression of respiratory rate or volume (3) The discharge frequency of the phrenic nerve was monitored in 2 dogs while respiratory arrest was induced by 175 mg /kg neomycin injected intravenously There was no decrease in frequency of discharge when apnea occurred and an increased discharge frequency was noted as anoxia ensued

Neostigmine antagonism to the curare like activity of neomycin was investigated in 3 dogs The minimum intravenous dose of neomycin required to produce apnea was found to be approximately 50 mg /kg At this dose the duration of respiratory arrest was 20 to 30 minutes Neostigmine methyl sulfate was given intravenously in doses of 50 to 100 μg /ml one to two minutes prior to the injection of neomycin and was found to elevate the dose needed to produce apnea by approximately 20% Pretreatment with neostigmine was noted to decrease the duration of respiratory arrest by approximately one third

The possibility that peritoneal trauma could allow a more rapid development of lethal serum levels through an increased rate of absorption was considered In 4 dogs with normal peritonea 200 mg /kg neomycin injected intraperitoneally caused no respiratory arrest The maximum neomycin blood level attained in these animals was 100 μg/ml occurring 30 to 40 minutes

after injection An equal number of dogs was subjected to peritoneal contamination with a fecal saline mixture and 8 to 12 hours later received 200 mg /kg neomycin intraperitoneally Each of these animals developed respiratory arrest 10 to 20 minutes after receiving the neomycin The average blood level at the onset of apnea was 200 μg /ml Four dogs without damaged peritonea were given 400 mg /kg neomycin intraperitoneally Two of these animals developed apnea 30 minutes following injection with peak blood levels of 200 μg /ml Maximum blood level in the 2 dogs which did not develop respiratory arrest never exceeded 100 μg /ml

Respiratory arrest of prolonged duration accompanied by persistent elevation of the neomycin blood level was noted to occur in 2 animals which developed an unexplained hypotension

SUMMARY

A progress report on a study of neomycin toxicity is presented It is concluded that neomycin does not cross the blood brain barrier in any appreciable concentration and effects no central respiratory depression Peritoneal damage allows for a more rapid absorption of the drug and definitely lowers the minimum lethal dose when used under such conditions Neostigmine has been noted to elevate the neomycin dose necessary to produce respiratory arrest and shorten the duration of the induced apnea Hypotensive conditions have been observed to intensify neomycin toxicity probably through decreased glomerular filtration

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RESPIRATORY ARREST FOLLOWING INTRAPERITONEAL ADMINISTRATION OF NEOMYCIN AN EXPERIMENTAL STUDY *

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Recent case reports [1 2] have pointed to a relationship between the administration of neomycin intraperitoneally and respiratory arrest In two patients

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with such a situation at this hospital the common factors were ether anesthesia relevant drugs and peritonitis This study attempts to evaluate these factors in the production of respiratory arrest following intraperitoneal neomycin

METHOD

Four groups of guinea pigs weighing 200 grams each (range 185 to 230 grams) received neomycin intraperitoneally The neomycin was in all cases diluted in 4 cc of saline A one hour period following the administration of neomycin was arbitrarily taken as the study time Respiratory arrests during this one hour period were attributed to neomycin

Group 1 animals were unanesthetized

Group 2 animals were anesthetized with ether to an anesthetic plane that made them relaxed and unresponsive to pain In general this plane was maintained for one hour following innoculation Anesthetized guinea pigs that did not receive intraperitoneal neomycin recovered uneventfully

Group 3 animals were unanesthetized Chemical peritonitis was induced by injecting toluene (25 cc diluted to 1 cc with saline) intraperitoneally One hour later the neomycin was administered Control animals in this group survived for approximately 24 hours

Group 4 animals were anesthetized with ether and subjected to laparotomy The animals were prepared with fecal peritonitis after the technique of Brown and Burnett[3] Peritonitis was established by excising an ellipse 3 mm in length from the wall of the colon The abdominal cavity was then closed Four hours later the animals were reoperated upon The elliptical defect in the colon wall was repaired using a 5 0 silk continuous suture Local contamination was removed by gentle swabbing and saline irrigation The neomycin was instilled in the abdominal cavity when closure was nearly complete Animals that appeared moribund at the time of reoperation were excluded from the study Approximately 100 control animals with peritonitis established in this way were treated with saline washes or other antibiotics to establish the technique and observe the type of death in guinea pigs with this operative procedure

Prostigmine was administered to an additional group of animals that received a dosage of neomycin previously shown to cause a high percentage of respiratory arrests in an attempt to ascertain the point of action of the neomycin An attempt was also made to test the potentiation of curare by neomycin We were unable however to establish a control group the response to curare being too variable

RESULTS

The response to the intraperitoneal neomycin was surprisingly constant Those animals that died showed in sequence 1) agitation 2) ruffling of the fur 3) muscular weakness and ataxia (in the unanesthetized group) 4) labored respiration and 5) respiratory arrest Initially during respiratory arrest the heart beat was regular and strong Artificial respiration by digital compression of the chest maintained this heart rate and apical pulse until this method of ventilation was abandoned In some animals this was continued up to one hour after the initial arrest No animal however survived once respiratory arrest occurred The interval between intraperitoneal innoculation and respiratory arrest appeared dependent upon the dosage of neomycin

Table 1 Summary of Results

NEOMYCIN ADMINISTERED (GRAMS)		4	3	25	2	15	1	075	05	025	0125	0063	0031	0013
Group 1	Total Animals	5	10	10	15	15	20	10	10		5			
	Number with Respiratory Arrest	5	9	8	14	10	4	0	0		0			
Group 2	Total Animals				10	15	10	10						
	Number with Respiratory Arrest				8	10	2	0						
Group 3	Total Animals						10	10			10			10
	Number with Respiratory Arrest						9	9			2			1
Group 4	Total Animals						5		7	5	3	5	10	8
	Number with Respiratory Arrest						5		4	4	2	0	3	3
Unanesthetized with Prostigmine	Total Animals		10	10	20	10	10							
	Number with Respiratory Arrest		9	6	7	4	1							

administered The larger the dose the quicker the arrest occurred: with peritonitis and the higher dosages of neomycin arrest occurred as rapidly as 2 minutes

The results are summarized in Table 1.

DISCUSSION

Large doses of neomycin were necessary to produce respiratory arrest in normal unanesthetized guinea pigs; ether anesthesia did not change this amount Chemical peritonitis decreased the amount of neomycin necessary to cause arrest. In animals with fecal peritonitis plus ether anesthesia and laparotomy, 1/77th of the control arresting dose of neomycin was sufficient to stop respiration It is presumed that the peritonitis allowed rapid absorption of a large quantity of neomycin, causing the toxic effect. The lowering of the percentage of respiratory arrest with prostigmine would suggest that neomycin acts as a local blocking agent similar to curare. We are not able, however, in a small group of animals, to reverse this arrest with prostigmine. Both patients who had respiratory arrest following intraperitoneal neomycin received large doses of prostigmine when they would not breathe spontaneously, it was thought at the time that they had been over-curarized The prostigmine had no apparent effect.

SUMMARY

1 An experimental study on the effects of intraperitoneal neomycin is presented

2 Large doses of neomycin are needed to cause respiratory arrest in the absence of peritonitis

3 Peritonitis markedly decreased the amount of neomycin necessary to cause arrest

4 There is some evidence that prostigmine will prevent the respiratory arrest, suggesting a curare like action of neomycin.

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THE EXPERIMENTAL PRODUCTION OF CARDIOSPASM IN DOGS *

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While performing studies on the effect of denervation of the heart it was found that esophageal dysfunction presented a major obstacle to long term survival of the dogs Consequently investigations were undertaken on the nature of the esophageal dyskinesia following autonomic denervation

Severe intractable vomiting following bilateral cervical vagotomy was observed by numerous investigators in the nineteenth century Kachkovsky[1] working in Pavlov s laboratory reviewed this subject extensively in 1899 The etiology of this vomiting was proven by Reider[2 3] to be constriction of the gastroesophageal junction at a time when vagotomy was advocated as the therapy for cardiospasm Hwan Essex and Mann[4] subsequently delineated the levels of vagotomy that result in cardiospasm in the dog and cat More recently Deloyers[5] *et al* have produced cardiospasm in cats by destruction of the autonomic ganglion cells in the terminal esophagus They used local injections of phenic acid Many experimental and clinical studies have helped to clarify the relationship of cardiospasm to dysfunction of the autonomic nervous system However the various forms of esophageal malfunction still present a challenge in diagnosis and therapy

METHOD

High cervical vagotomy was performed on 20 dogs These dogs were then followed with esophagograms and upper gastrointestinal series Pylorotomy was performed on all dogs to obviate the effects of pylorospasm Preliminary studies revealed a high early mortality rate due to aspiration pneumonia For this reason laryngectomy was performed in 14 dogs and cervical esophagostomy in 6 dogs During the postoperative period nutrition was maintained by parenteral fluids gastrostomy feedings and esophageal intubation

In 9 dogs esophagocardiac myotomy was performed through the thorax 3 to 10 days following vagotomy A 4 cm myotomy incision was used To insure that all muscle fibers were severed the incision was carried through the mucosa in 3 dogs and the longitudinal incision was closed in a transverse fashion Resection of the gastroesophageal junction was performed in 3 dogs including 4 cm of the distal esophagus and the cardia of the stomach The esophagus was then anastomosed to the remaining stomach

In 6 dogs the gastroesophageal junction was observed following acute vagotomy Evaluation of the effect of myotomy was made by use of a hydrostatic system in 3 dogs using a reservoir connected to a Foley catheter with

* From the University of Minnesota Medical School and Hahnemann Medical College and Hospital Supported by grants from the Heart Association of Southeastern Pennsylvania and USPHS 5634 2800

the balloon distended in the proximal esophagus Intraluminal pressures were measured in 3 dogs by 3 polyethylene catheters with their openings 1 cm apart and connected to 3 Statham strain gauges

Bilateral total thoracic and lumbar sympathectomy was done on 4 dogs prior to cervical vagotomy These dogs were then followed with x ray studies before and after vagotomy

RESULTS

Esophageal dysfunction was present immediately following high bilateral cervical vagotomy When the dogs recovered from anesthesia, they vomited all fluids and foods taken by mouth Esophagograms made without anesthesia on the first postoperative day revealed a constriction at the gastroesophageal junction and a mild dilatation of the terminal esophagus Very little barium was seen to enter the stomach after 2 hours No normal peristaltic waves were noted at the time of fluoroscopy Only small, ineffective, intermittent, segmental movements were observed Followup studies were made to a period of 28 postoperative days The cardiospasm was never observed to relent during this period of time The esophagus became progressively more distended and tortuous (Fig 1 and 2) The dogs were permitted to ingest the normal diet although this food was inevitably vomited When reingested, the food was repeatedly regurgitated Without adequate supplementary nutrition the dogs became emaciated and weak and succumbed

The x ray studies revealed the area of constriction to be limited to approximately the distal 1 5 to 2 cm of the esophagus Therefore, it was a surprise to discern persistent constriction of the distal esophagus following myotomy and gastroesophageal resection In 6 dogs, reoperation was performed and the myotomy was extended 2 to 3 cm proximal to the initial incision The dogs did not tolerate this final procedure well and died on the first to sixth postoperative days However, the signs of esophageal dysfunction persisted and esophagograms revealed constriction of the distal esophagus

To further elucidate the nature of this distal area of constriction, the esophagus was exposed and the function was observed directly either with a

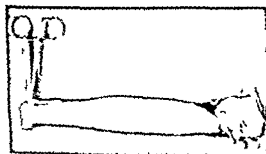


Fig 1 Esophagus and cardiac portion of stomach taken from dog 34 days following bilateral cervical vagotomy The esophagus has been filled with water Even in this post mortem specimen there is little tendency for the water to pass through the gastroesophageal junction Note the distention of the esophagus and the constriction of the gastroesophageal junction The arrow points to the constricted orifice of the esophagus



Fig 2 This esophagogram was made 28 days following bilateral cervical vagotomy The esophagus was filled with a thin suspension of barium and x rays were taken 30 60 and 120 minutes later Note the dilation and tortuosity of the esophagus Very little barium suspension entered the stomach even after 2 hours

out with water thereafter Penicillin and streptomycin were administered subcutaneously for 5 days

RESULTS

Three of the dogs died within the first 3 postoperative days Autopsy revealed no specific reason for these deaths and they were attributed to anesthesia postoperative depression and possibly shock One of the dogs died of pneumonia on the twenty fifth postoperative day Three more dogs died between the second and the fourth weeks as a result of electrolyte imbalance from leaking gastrostomy tubes These animals repeatedly chewed off the tip of the gastrostomy tube leading to considerable loss of stomach juices inanition and death In no instance did a dog die of mediastinitis

In most instances by the third and fourth day the animals were alert showed an interest in their surroundings and sought attention when someone approached the cage

In 7 to 14 days the neck fistula was healed In one instance spontaneous tubulation was complete by the fourteenth day Oral feedings were started between the fifteenth and twenty fifth days Early during the experiment the gastrostomies were maintained for as long as 49 days but later it was found that the dogs progressed more favorably if the gastrostomies were closed between the fifteenth and twenty fifth days or as soon as the animals showed ability to take adequate amounts of food by mouth

Esophagrams were taken at 15 days 30 days and at 2 3 and 4 months An adequate tube was demonstrated in some of the dogs as early as the fifteenth day There appeared to be no dysfunction There were no areas of stricture It was difficult or impossible to distinguish the operated segment of esophagus from the remaining portion of this organ

Autopsies showed that the esophageal strip had formed a completely closed tube continuous with the remaining segments of the esophagus The operated segment was somewhat narrower than the esophagus distal to the point of surgery The newly formed tube however was soft and easily distensible There were no areas of stricturing fibrosis or abscess formation

Histological sections from these newly formed tubes were studied at 25 days 3½ months and 5½ months

The sections again showed a somewhat narrowed caliber of the retubulated area when compared with the normal esophagus distal to the operative site The lumen of the tube was completely lined with squamous epithelium The muscularis mucosa of the retubulated portion was circumferentially intact The submucosa and muscular fibers of the esophagus could be identified clearly throughout 95% of the newly formed tube In one small area which represented 5% of the entire circumference the mucosa and muscularis mucosa layers were intact the submucosa and muscle layers were lacking being replaced by connective tissue

CONCLUSIONS

It was found that a narrow open strip of cervical esophagus in the dog will undergo spontaneous tubulation This will occur within 15 to 25 days providing the nutritional status of the dog can be maintained At the end of this period the dog can swallow without difficulty and maintain adequate nutrition At autopsy the narrow strip of mucosa has spontaneously developed into a completely closed tube continuous with the remaining portions of the

esophagus These tubes though somewhat smaller in caliber than the remaining portions of the esophagus are soft and pliable and easily distensible Histological sections through these tubes show concentric layers of mucosa muscularis mucosa The submucosal and muscle layers encircle 95% of the re established lumen but the remaining 5% consists of connective tissue These findings may help explain the healing powers of the human esophagus and pharynx

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IMMEDIATE RECONSTRUCTION OF THE CERVICAL ESOPHAGUS BY A REVASCULARIZED ISOLATED JEJUNAL SEGMENT *

Bernard Seidenberg and Elliott S Hurwitt

Surgical treatment of carcinoma of the hypopharynx and cervical esophagus has been hindered by the difficulty in completely extirpating the lesion with a wide margin of safety and following this with a reliable one stage reconstruction of the pharyngoesophagus To date the procedures employed for cervical esophagus reconstruction leave much to be desired either because they are multistaged and create a pharyngostome (Wookey [1, 2]), or they predispose to the development of pharyngeal leaks and early strictures when a split thickness skin graft is used [3, 4, 5, 6, 7]

An experimental study was undertaken to develop a one stage immediate reconstruction technique utilizing a free jejunal segment completely isolated from its mesenteric blood supply The cervical esophagus was resected in dogs and immediately replaced with a free jejunal segment which was revascularized in the neck

METHOD

Mongrel dogs weighing from 30 to 75 lbs were used All operations were performed under general anesthesia produced by a veterinarian solution of nembutal administered intravenously

Separate cervical and abdominal incisions were used in each experiment A midline incision was made in the neck from the suprasternal notch to the hyoid bone with a left lateral limb at the upper end A skin and platysma flap was developed exposing the trachea medially and the external jugular vein laterally The cervical vessels to be utilized for revascularization of the free jejunal segment were then dissected cleanly from the surrounding tissues and mobilized The vessels employed were the superior thyroid artery and the anterior facial vein The sternocleidomastoid muscle and the carotid sheath

* From the Surgical Division the Montefiore Hospital New York City Work done in the Henry and Lucy Moses Research Laboratories Aided by a grant from the A Shapiro Surgical Research Fund with the technical assistance of Ruthven Ferreira and Alphonso Ivan Henry

contents were retracted laterally and the cervical esophagus was mobilized After hemostasis was achieved, the entire circumference of the cervical esophagus was resected from 1½ cm distal to the cricoid cartilage to the suprasternal notch

A left paramedian muscle splitting abdominal incision was made A segment of jejunum supplied by an acceptable radial branch of the superior mesenteric artery and vein was chosen for free transplant to the neck The base of the mesentery in the region of the chosen artery and vein was infiltrated with 2 cc of 1% procaine solution in order to decrease the amount of vasospasm produced by dissection of the vessels A 2½ in segment of jejunum with its mesentery containing the previously mobilized artery and vein was removed from the peritoneal cavity The continuity of the intestinal tract was reestablished by an end to end jejunojejunostomy The isolated jejunal segment was immediately perfused with heparin solution injected into the mesenteric artery in order to remove the residual blood and to prevent thrombosis anywhere in the resected specimen The mesenteric vein was prepared for anastomosis to the anterior facial vein by evaginating it onto a highly polished, siliconized tantalum ring prosthesis This was done with the aid of 3 traction sutures of 6 0 arterial silk placed in the transected end of the vein The vein was passed through an appropriate sized ring usually measuring 2 to 2½ mm and then rolled onto the outer surface of the ring It was fixed to the ring with a 4 0 silk tie secured on one of the two ridges on its outer surface

The jejunal segment was then implanted into the defect in the cervical esophagus The posterior half of the proximal and distal esophagojejunal anastomoses were completed before the segment was revascularized This was done before the revascularization in order to stabilize the segment so that the vascular anastomoses would not be compromised by undue manipulation The esophagojejunal anastomoses were performed by the Cameron Haight technique[8] The venous anastomosis was then completed by attaching the proximal transected end of the anterior facial vein to the mesenteric vein over the tantalum ring prosthesis Again, 3 traction sutures of 6 0 arterial silk were placed in the cut end of the vein and they were used as guides to slip the anterior facial vein over the tantalum ring A 4 0 silk tie placed over the second ridge on the ring surface fixed the vein to the ring and to the mesenteric vein (Fig 1 A, B, C) The distal transected end of the mesenteric artery and the proximal end of the superior thyroid artery were then anastomosed by the continuous over and over suture technique using 12 in lengths of 7 0 braided silk sutures, armed at each end with an 008 in wire, ⅜ circle, taper point, round atraumatic needle (Fig 1 A, D, E) †

Upon completion of the arterial anastomosis, the isolated jejunal segment was revascularized and immediately became pink and displayed peristalsis The venous anastomosis acted as an adequate runoff for the new blood supply and the viability of the jejunal segment was maintained The anterior halves of the proximal and distal esophagojejunal anastomoses were then completed (Fig 2 A, B) Before the neck incision was closed, the mobilized anterior facial vein was secured to the surrounding tissue by a carefully placed 4 0 silk suture

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† *Supplied through the generosity of the Ethicon Suture* Laboratories Inc New Brunswick, N J

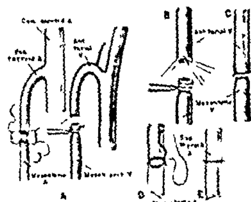


Fig 1 A D & F) Demonstrate the anastomosis of the superior thyroid artery to the mesenteric artery by the continuous over and over suture technique A B & C) Demonstrate the attachment of the anterior facial vein to the mesenteric vein over the tantalum ring prosthesis

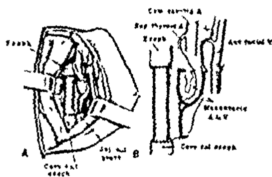


Fig 2 A) The isolated jejunal segment is revascularized and the esophagojejunal anastomoses are completed B) A more detailed diagram demonstrating the complete reconstruction of the cervical esophagus by a revascularized isolated jejunal segment

through its adventitia so that it would not kink and obstruct the venous run off The abdominal and neck incisions were closed The neck incision was drained at its inferior angle In order to protect the vascular anastomoses from disruption during the excitement phase of recovery from the anesthesia, the dogs were placed in a brace that completely immobilized the head and neck The brace was removed 24 hours later

Postoperatively the dogs were maintained on penicillin, streptomycin, and achromycin intramuscularly, 1 000 cc of 5% dextrose in saline intravenously daily, and nothing by mouth for 5 to 7 days If there was no leakage of mucous through the cervical incision within 5 days, the reconstructed cervical esophagus was viable and the dog was fed fluids and ground meat orally After 10 days the dogs were fed regular kennel rations and maintained their nutrition For several months they were disturbed by the accumulation of mucous in the pharynx and would expectorate moderate amounts of mucous early in the feedings following which they swallowed without difficulty

RESULTS

This experimental study cannot be analysed statistically because numerous changes were made throughout its course until the most effective method of immediate esophageal reconstruction by revascularizing a free jejunal segment was developed The technique described represents the final product of the study Careful analysis of each failure suggested changes that eliminated flaws The factors most important for success were 1) the development of the non reactive tantalum ring prosthesis for the venous anastomosis, 2) the use of meticulous technique and 7 0 arterial silk sutures with .008 in needles for the small arterial anastomoses 3) utilizing a brace that effectively stabilized the head and neck and prevented disruption of the vascular anastomoses during the early postoperative excitement period

Four long term survivors were obtained ranging from 14 to 27 months The free jejunal segment was without blood supply in the survival experiments for a minimum of 1 hour and a maximum of 2 hours Esophagram by barium

swallow revealed good patency of the reconstructed cervical esophagus Carotid angiography 1 to 2¼ years postoperatively on all survivors demonstrated the patency of the recreated arterial and venous circulation of the isolated jejunal segment

After sacrifice, the revascularized jejunal transplants were studied grossly and microscopically The jejunal anatomy was preserved even after 27 months in the location of the cervical esophagus The mucosa remained jejunal but was somewhat atrophic

DISCUSSION

The advantages of reconstruction of the cervical esophagus by a revascularized isolated jejunal segment are that it avoids the objectionable features of the various techniques employed to date It is a one stage operation permitting wider excision with combined neck dissection No pharyngostome is created Early strictures and the high incidence of pharyngeal leaks that may accompany the procedures utilizing split thickness skin grafts are eliminated

This technique, however, requires considerable experience and dexterity in vascular surgery The technique of anastomosing small arteries as done in this laboratory, was developed during experimental studies involving over 300 such anastomoses since 1950,[9, 10, 11, 12] and has been described in detail in a previous publication[13]

The venous anastomosis required a rigid nonreactive, ring prosthesis to maintain its patency because the flaccidity and low pressure in small veins prevented successful suture anastomosis Highly polished tantalum rings that were carefully defatted and siliconized proved to be a reliable prosthesis that did not stimulate tissue reaction resulting in venous thrombosis

SUMMARY

1 Restoration of pharyngoesophageal continuity upon completion of removal of tumors of the hypopharynx and cervical esophagus represents one of the major challenges in this type of surgery

2 A one stage immediate reconstruction of the cervical esophagus by an isolated jejunal segment revascularized in the neck has been developed in dogs

3 The arterial and venous aspects of the operative technique are described in detail

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GASTRIC SECRETION IN THE FASTING STATE AND AFTER ANTRAL STIMULATION IN PATIENTS WITH CIRRHOSIS AND WITH PORTACAVAL SHUNTS*

JAMES S CLARKE ROBERT COSTARELLA AND SYLVESTER WARD

The incidence of benign gastroduodenal ulceration in patients with cirrhosis of the liver is higher than that in the general hospital population Fainer and Halsted[1] found peptic ulcers in 17% of 91 patients with cirrhosis and peptic in 10 6% of 1,181 other cases without cirrhosis The literature bearing on this point has been summarized recently by Koide Texter and Borden[2] The reported frequency of ulcers in cirrhotics varies from 3 6% to 21 7%

It is not known whether ulcers occur with increased prevalence after portacaval shunt However Dubuque Mulligan and Neville[3] observed upper gastrointestinal ulceration in 15% of 60 patients followed up to 8 years after end to side portacaval anastomosis Out of 62 patients with portacaval shunts we[4] found 5 who developed ulcers within 9 months after operation and 4 of the 5 had no previous ulcer history In other reports of followup on patients with portacaval shunts sporadic cases of postshunt ulcers have been noted

We[4] have confirmed the findings of others[3, 5, 6, 7] that the acid secretion from Heidenhain gastric pouches of dogs is profoundly increased when the portal venous blood is diverted around the liver, and that this increase is intimately related to the ingestion of food Whether there is an abnormality of gastric secretion in patients with cirrhosis or with portacaval shunts remains unsettled Studies on gastric secretion in cirrhosis have been few, and those summarized by Lebedinskaja[5] were not conclusive Patients with cirrhosis have a varying degree of collateral circulation of their portal venous blood around the liver and in patients with operative shunts a significant volume of portal blood bypasses the liver Do these perihepatic shunts in humans result in an elevation of gastric acid secretion and consequent ulceration or is the hypersecretion of acid from canine gastric pouches after shunt a false lead in attempting to explain the clinical observations?

METHOD

Gastric secretory studies were done on 3 groups of adult male patients Group 1 consisted of patients with no signs of cirrhosis of the liver by history, physical or laboratory examination They were hospitalized for surgical conditions other than gastroduodenal ulcer and were not acutely ill or chronically debilitated Group 2 had clearly diagnosed cirrhosis of the liver on the basis of clinical and laboratory evidence Group 3 was composed of patients who had portacaval shunts The average age of the 43 patients in Group 1 was 46 2 years (range 23 to 71) of the 42 patients in Group 2 52 0 years (range 31 to 83) and of the 8 in Group 3 48 3 years (range 39 to 68)

Three tests of gastric secretion were employed The first was measurement of acid secretion from the stomach of patients in the basal fasting state A

* From the Surgical Service Veterans Admin Center and the Dept of Surgery University of California Medical Center Los Angeles Supported by research grant H 3066 from the National Heart Institute Public Health Service

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Levin tube was placed in the *gastric antrum* under fluoroscopic control at 8 a.m. after a 14 hour fast and the stomach was emptied. Then the gastric output was aspirated continuously utilizing a pump for a 2 hour period, an effort being made to avoid contamination with saliva. The volume was measured and the free acidity determined by titration with 0.1 N NaOH to the color change of Toepfer's reagent. The HCl output was calculated and expressed as mEq/hour.

Secondly, we measured the gastric response to a test "meal" of 750 ml of 3.5% sucrose in water with phenol red as described by J. N. Hunt.[8] Determination of the volume, acid, chloride and phenol red concentrations of the gastric content at 30 minutes after giving the test meal allows one to calculate the output of acid, chloride, volume of parietal and nonparietal components, and volume of gastric content passing the pylorus in ½ hour. All tests were discarded in which the aspirate was bile stained or the volume of gastric content at ½ hour was less than 250 ml. This test meal presumably stimulates gastric secretion primarily through distention of the gastric antrum.

Thirdly, we fed patients an appetizing breakfast of 180 ml. of orange juice, cereal, 2 eggs, 1 slice of toast, 3 strips of bacon, 10 gm. of butter, 240 ml. of milk, and 6 gm. of sugar. This contained 31.4 gm. protein, 25 gm. fat and 74 gm. of carbohydrate. A Levin tube was passed ½ hour after the meal was eaten, and samples were withdrawn at ½ hour intervals for 4 to 6 hours and analyzed for free acid. The acid output after this meal includes cephalic and intestinal as well as the gastric phase of secretion.

RESULTS

The findings are presented in Table 1. The basal secretion was on the average lower in patients with cirrhosis or shunt than in patients without these conditions, but this difference was not statistically significant at the $\alpha = .05$ level by the "t" test.[9]

Table 1. Results of 3 Types of Secretion Test in 3 Groups. Values are mean averages. Numbers of patients tested are given in parentheses.

	1 CONTROLS	2 CIRRHOTICS	3 POSTSHUNT
1. Basal acid secretion (mEq./hr.)	1.39 (30)	0.88 (30)	0.[illegible]7 (8)
2. Hunt test			
Acid output (mEq.)	18.45 (16)	23.73 (8)	22.06 (2)
Chloride output (mEq.)	90.66	89.85	105.42
Parietal component (ml.)	231.02	213.11	271.50
Nonparietal comp. (ml.)	807.06	426.40	473.50
Vol. gastric content passing pylorus in ½ hour (ml.)	825	787	809
3. Acidity after Meal (mEq./l.)	44.08 (13)	37.70 (21)	62.33 (3)

There was no sharp difference between cirrhotics and noncirrhotics in any of the 5 parameters calculated from the Hunt test, except that the volume of nonparietal component was reduced in cirrhotics This latter difference was significant at the $\alpha = .05$ level by the 't' test The results of the 2 tests on postshunt patients were in the range of the cirrhotic group Although the calculated volume of gastric content passing the pylorus was similar in all 3 groups 23 of 31 Hunt tests (74 1%) in cirrhotics and 8 of 10 (80%) in postshunt patients had to be discarded because the gastric content at ½ hour was less than 250 ml whereas only 14 of 30 (48 7%) were unsatisfactory for this reason in the normal group Thus gastric emptying was usually more complete at ½ hour after introduction of the test 'meal' in cirrhotics and postshunt patients than in the others

The maximum concentration of free acid attained after eating the test breakfast was similar in the cirrhotic and noncirrhotic group The 3 postshunt patients achieved a higher acidity of the gastric content after the test breakfast than was usual in the other 2 groups but their numbers are insufficient for any firm conclusions

DISCUSSION

The increased secretion of acid from the stomach or from accessory gastric pouches of dogs with perihepatic portal venous shunts is closely related to eating[5, 6, 7] and is reduced during fasting[4] Available evidence suggests that this increase is due to a humoral secretagogue which originates in the organs of the portal bed and is normally inactivated in the liver Gregory[7]
to histam
after port
and Weiss[6] found that pouch secretion of acid after a meal is markedly elevated and prolonged after portacaval shunts Thus each of the 3 phases of gastric secretion might be increased also in humans with the perihepatic venous circulation from portal collaterals in cirrhosis

Utilizing these 3 tests of gastric secretion we have not detected any marked differences between the 3 groups of patients The basal fasting studies give no evidence for hypersecretion of acid on a neurogenic basis The Hunt tests demonstrated no elevated secretion of acid during the gastric phase of secretion induced by distention of the pyloric antrum The feeding tests in which all 3 phases of gastric secretion come into play give no data to support the idea that there is abnormal acidity in the stomach of cirrhotic patients after eating Thus none of these tests unveiled an elevation of acid secretion like that seen in dogs with shunts More data are needed especially in the postshunt group with tests 2 and 3 for the numbers are not sufficient for firm conclusions The possibility of an abnormal response to stimulus from gastrin or intestinal secretagogues has not been adequately explored by the studies reported here

SUMMARY

Three types of tests of gastric acid secretion were done on patients without cirrhosis or ulcers patients with cirrhosis and patients with portacaval shunts Basal fasting secretion in the cirrhotic and postshunt groups was not significantly different from the controls There was no marked difference

between cirrhotics and controls in the response to swallowing 750 ml of 3 5% sucrose and to eating a regular breakfast

We are grateful to Dr J N Hunt for helpful advice

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EXPERIMENTAL PRODUCTION OF ATROPHIC GASTRITIS *

EDWARD H STORER FRANCIS C NANCE DAVID T DODD
JOE CAMPBELL AND ROBERT A PARRISH JR

Atrophic gastritis is of considerable importance because of its frequent association with gastric cancer In addition gastric atrophy invariably accompanies pernicious anemia The etiologic relationship between these diseases and gastric atrophy remains obscure Atrophic gastritis gastric carcinoma and pernicious anemia are uniquely human diseases and rarely if ever occur spontaneously in animals The need for a method of producing gastric atrophy in the experimental animal has repeatedly been recognized but no consistent reproducible method has yet been reported though some progress has been made [1 2 3 4]

The following is a preliminary report of production of sustained partial atrophy of the gastric mucosa in the dog by means of repeated hot water injury It is expected that consistent complete permanent gastric atrophy will soon be attained

METHOD

Heidenhain pouches were produced and daily collections of the pouch secretions made using the cannula technique of Dragstedt [5] Secretory data are reported as mEq of HCl produced per 24 hour period Another parameter of secretory function is the pouch response to histamine This is measured as mEq of HCl produced in the 70 minute period following a single subcutaneous injection of 6 times the normal dose of histamine base (calculated on a body weight basis —0 1 mg /10 lbs) combined with 25 mg of an antihistamine This augumented histamine test is designed to yield a maximal stimulus to the parietal cells [6]

* From the Department of Surgery University of Tennessee College of Medicine Memphis Supported by research grants (RG 1469 and C2950) from the National Institutes of Health

Gross and microscopic morphology of the pouch mucosa are also observed The lumen of the pouch is examined by means of an endoscope which is essentially a modified baby proctoscope Biopsies of the pouch mucosa are taken through this scope using a uterine biopsy forceps Occasionally, more generous biopsy specimens are obtained by laparotomy Biopsies are fixed in buffered formalin and stained with the routine H & E stains

Several methods of instilling hot water into the pouch have been tried A major problem has been to get hot water in fast enough to prevent a large temperature drop since the gastric wall is a very effective heat exchanging mechanism Early trials with manual instillation using large syringes were only partially effective A sigmamotor pump set to deliver 2000 cc /min is now used The hot water is pumped in through a catheter inserted into the pouch through the cannula Free egress of the hot water is allowed around the catheter Distension of the pouch is maintained by the high flow rate of the pump There is less than 1°C temperature drop during passage of the water through the pouch

RESULTS AND DISCUSSION

Twenty four Heidenhain pouches were standardized by measuring their daily secretions for at least 1 month usually longer and by determining the quantitative histamine response in most cases Following this the pouches were exposed to a sufficient number of hot water irrigations to produce achlorhydria Several different hot water temperatures were tried ranging from 55° to 75°C Several different irrigation times also were tried ranging from 20 sec to 15 min Using 55° water for 15 min resulted in the deaths of 9 of the animals in from a few hours to 2 months Autopsy showed that the burn necrosis had included the entire pouch wall with death from perforation Overdistension from faulty perfusion technique may have played a role in the perforations It is now felt that very hot water for a very short time will give the most uniform and also the most desirable superficial necrosis 71°C water perfused for 30 sec is now being used

Fourteen animals have survived for a sufficiently long period for significant data to be obtained These are summarized in Table 1

Three patterns of response to burning have been noted An insufficient burn is manifested by early return of acid secretions first to histamine followed by free acid in the daily secretions The histology of the pouch mucosa parallels the secretory findings in that a more or less normal mucosa is found somewhat before return of function Dog 150 is an example of such a burn

Overburning usually results in death but occasionally the pouch is walled off by surrounding viscera and peritonitis thus prevented Dog 117 is an example of an overburn The Heidenhain pouch in this animal scarred to a tiny remnant with a cavity just large enough to accommodate the cannula flange

Adequate thermal injury is exemplified by dog 23 (see Fig 1 2)

Our observations of the response of the gastric mucosa to thermal trauma do not differ significantly from the observations of others using other types of injury [3, 4] The advantages of this method are several It is reproducible easily controlled may be repeated as necessary to maintain achlorhydria and the animals remain in good health The progress of the lesions produced

Table 1. Duration of Depression of Secretory Function of Heidenhain Pouches Following a Hot Water Burn Series to the Pouch Mucosa

DOG #	DURATION OF "DAILY ACHLORHYDRIA	DURATION OF HISTAMINE ACHLORHYDRIA	HYPOCHLORHYDRIA DURATION	HYPOCHLORHYDRIA % OF CONTROL	REMARKS
23	9 mo	9 mo	23 mo →	6%	
27	1 mo		23 mo →	20%	
41	3 mo		22 mo →	18%	
63	1 week		10 mo	40%	reburned
	1 week		3 mo	47%	reburned
	6 mo →	6 mo →			
117	15 mo	15 mo			pouch fibrotic
121	3 mo →	3 mo →			
122	8 mo	3 mo	13 mo	100%	reburned
141	6 mo →	6 mo →			
150	1 mo	1 week	2 mo	100%	reburned
	2 mo →	2 mo →			
153	4 days	4 days	3 mo	27%	reburned
	6 mo →	6 mo →			
170	6 mo →	6 mo →			
171	6 mo →	6 mo →			
178	6 mo →	6 mo →			
180	3 mo →	3 mo →			

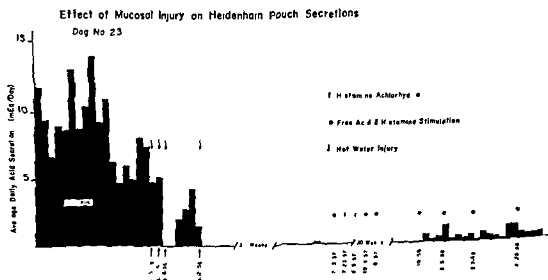


Fig 1 An example of adequate thermal injury to the pouch mucosa Note long period of achlorhydria following burn series Hypochlorhydria has been maintained through 8/30/58 (Each bar represents a period of 1 week)

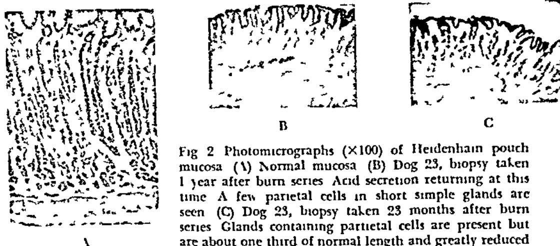


Fig 2 Photomicrographs (×100) of Heidenhain pouch mucosa (A) Normal mucosa (B) Dog 23, biopsy taken 1 year after burn series Acid secretion returning at this time A few parietal cells in short simple glands are seen (C) Dog 23, biopsy taken 23 months after burn series Glands containing partietal cells are present but are about one third of normal length and greatly reduced in number An exuberant mucus secreting columnar epithelium covers the surface

are easily followed by direct observations by endoscopy, and by biopsy, as well as by functional studies of the HCl secretion

SUMMARY

An experimental method of inflicting controlled thermal injury to the mucosa of a Heidenhain pouch is described Achlorhydria lasting several months is produced and can be maintained by repeated injury The histologic change produced resembles gastric atrophy seen in humans

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MODIFICATION OF GASTRIN MECHANISM BY ANTRONEUROLYSIS *

ROBERT V DEVITO, THOMAS W JONES, ANDREW J MARTINIS, LLOYD M NYHUS, AND HENRY N HARKINS

The pyloric antrum plays a prominent role in gastric secretion by liberating, in response to diverse stimuli, a hormone called gastrin which stimulates production of hydrochloric acid from parietal cells It has long been established that application of cocaine or atropine solutions to the mucosa of the antrum completely blocks normal secretory responses to antral stimuli,

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Supported by U S Public Health Service Grant, #RG 3479

suggesting that neural mechanisms are involved in the release of gastrin Recently, it has even been suggested that the "gastrin cell" (as yet not identified) might be a modified nerve cell[1] Since section of all extrinsic nerves to the antrum does not abolish secretory responses to antral stimulation, an investigation of the role played by the intrinsic neural elements was developed This study involved a new operative procedure, termed antroneurolysis, designed to disrupt structures of the submucosal nerve plexus The operation consists essentially of circumferential sharp dissection in the submucosal plane of the entire pyloric antrum[2]

METHOD

The experiments were performed on 17 mongrel dogs, 14 with Heidenhain and 3 with Pavlov pouches Pouch secretions were collected each 24 hours and analyzed The results in all cases express the average daily free hydrochloric acid secretion over a minimum period of 30 days collections following full recovery from each operation

The initial operative procedure in all dogs was the formation of a gastric pouch After adequate baseline collections, all of the Pavlov and 12 of the Heidenhain animals had antroneurolysis performed This consisted of a submucosal dissection of the entire antrum, accomplished through a 6 to 8 cm incision in the midanterior wall of the antrum, parallelling the greater curvature Care was taken to preserve extrinsic innervation and the anastomotic submucosal blood vessels and to avoid entrance into the lumen

Of the Heidenhain pouch dogs with antroneurolysis, 3 subsequently had large stoma gastrojejunostomies performed, 2 had bilateral thoracic vagotomies, and 4 had antral transplants to the transverse colon The 2 remaining Heidenhain pouch animals underwent bilateral thoracic vagotomy as the second, and antroneurolysis as the third, phase

RESULTS

The animals tolerated the antral dissection well and in no instance did the antroneurolysis produce complications The antrum mucosa remained grossly unremarkable, and histology 1 month following the procedure showed microscopically intact epithelium At subsequent operations, there was no evidence of outlet obstruction, and peristalsis persisted in the pyloric canal Electrical stimulation of the vagi produced good contractions of the antral musculature

The 12 Heidenhain pouch dogs secreted an average of 20 8 mEq of HCl per 24 hours Following antroneurolysis, secretions averaged 9 8 mEq /day, for an average decline of 53% (Fig 1) The longest followup in this series is 9 months and none of the animals have shown a subsequent rise in secretion

The 3 Pavlov animals responded differently Antroneurolysis increased secretions in 2 animals, to 130 and 146% of baseline levels, and produced no change in the third

When preceded by antroneurolysis gastrojejunostomy produced a slight rise in secretion averaging 28% above antroneurolysis levels, but the final level of secretion remained at or below baseline Heidenhain pouch secretions (Fig 2)

Antroneurolysis likewise modified the usual secretory response to vagotomy (Fig 3) When vagotomy was performed subsequent to antroneurolysis (dogs

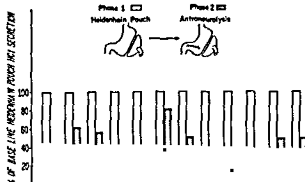


Fig 1 The effect of antroneurolysis upon 24 hour Heidenhain pouch secretion For each dog the average 24 hour pouch secretion of free HCl is expressed as the percentage of the average control secretion The average depression for the group as a whole was 53% below the control levels

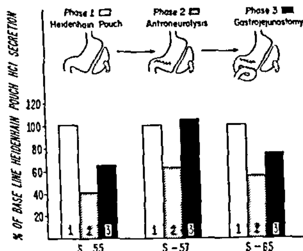


Fig 2 The individual responses of 3 dogs to gastrojejunostomy (4 cm stoma) following antroneurolysis In phase 3 the secretions average 19% below the baseline Heidenhain pouch output (phase 1)

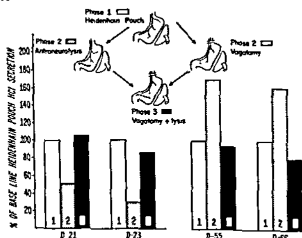


Fig 3 *Experiments on 4 dogs demonstrating* the effect of antroneurolysis on Heidenhain pouch secretions when accompanied by vagotomy In dogs D 21 and D 23 the antroneurolysis preceded the vagotomy in the other pair antroneurolysis was the final phase Note that the usual hypersecretory response to vagotomy is markedly reduced by antroneurolysis For the entire group secretions in the final phase approximated *baseline levels* averaging 92% of baseline free HCl output per 24 hours

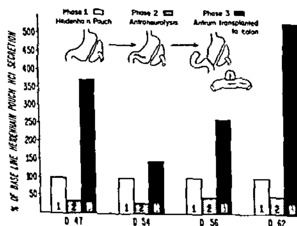


Fig 4 Individual experiments on 4 dogs which underwent transplantation of the antroneurolysed antrum to the colon as a diverticulum The striking *increase in secretion* averaged 325% over Heidenhain pouch controls

D 21 and D 23), the ultimate level of secretion approximated baseline values In dogs D 55 and D 66, antroneurolysis abolished the moderate hypersecretion produced by thoracic vagotomy

Despite previous antroneurolysis which had depressed Heidenhain pouch secretion from 50% to 70%, transplantation of the antrum to the colon in 4 dogs produced marked hypersecretion averaging 325% of baseline acid output (Fig 4) One dog (D 47) had a perforated peptic ulcer 76 days following transplantation

DISCUSSION

Antroneurolysis significantly reduces Heidenhain pouch secretion, when the antrum is retained in normal continuity, presumably by depressing gastrin release Inasmuch as nerve blocking agents such as cocaine applied to the antrum mucosa have a similar effect, it is logical to conclude that antroneurolysis attenuates gastrin release by disruption of local nervous reflexes within Meissner's plexus The effect on Pavlov pouches is in the opposite direction—secretions rise above baseline levels This suggests that the vagal mechanism of secretion is released from some form of inhibition which is dependent upon intact myenteric plexuses These data require more confirmation, however, before definite conclusions are expressed

The failure of gastrojejunostomy or vagotomy to produce Heidenhain pouch hypersecretion when antroneurolysis is also done further supports the concept that the stimulation of secretions usually seen following these procedures is secondary to augmentation of antral mechanisms [3, 4]

Antroneurolysis does not completely abolish the antral phase of secretion indeed, when the antrum is stimulated strongly (as a diverticulum transplanted to the colon) very high levels of gastrin production are attained This discrepancy between the activity of the antrum after antroneurolysis when left *in situ* as compared to its activity as a colon transplant may be explained as follows 1) antroneurolysis elevates the threshold of the gastrin release mechanism to normal stimuli, 2) antroneurolysis sensitizes the gastrin cell to acid inhibition of hormone release That is, following disruption of Meissner's plexus stronger stimulation is required to provoke gastrin release and/or the 'turn off' effect of acid on the gastrin mechanism is potentiated Preliminary studies on irrigation of the isolated, antroneurolysed antrum indicate that the response of the antrum to acid solutions is not reduced by antroneurolysis Heidenhain pouch secretory responses elicited by strong antral distention stimuli are promptly abolished by perfusion of the antrum with acid at pH 1

The concept that develops from the foregoing considers that cells within the antral mucosa secrete gastrin in response to certain stimuli, antroneurolysis removes a facilitatory mechanism resident in Meissner's plexus but does not abolish the inhibitory effect of acid on gastrin release The gastrin mechanism is therefore reduced when the antroneurolysed antrum is in normal continuity because the threshold for response has been raised and because acid is present Gastrojejunostomy increases secretion slightly because antral stimulation is increased and/or regurgitation of alkaline juices tends to neutralize the inhibiting acid Vagotomy likewise produces a slight rise in secretion over antroneurolysis levels, by increasing antral stimulation while decreasing acid production from the main stomach When the antroneurolysed antrum is transplanted to the colon as a diverticulum the combination

of very strong antral stimulation and no acid result in brisk activation of the gastrin mechanism and a copious secretory response occurs

SUMMARY AND CONCLUSIONS

1 Surgical disruption of the submucosa of the gastric antrum (antroneurolysis) depresses Heidenhain pouch secretion of average of 53%

2 Circumstances which increase antral stimulation and raise antral pH (gastrojejunostomy vagotomy or antral transplant to the colon) demonstrate that antroneurolysis does not abolish the capability of the antrum to produce gastrin

3 Antroneurolysis is considered to reduce facilitatory influences normally mediated by the submucosal plexus of Meissner so that the threshold of gastrin producing cells in the antral mucosa to normal stimuli is elevated

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THE CAUSE OF FATALITIES IN BILATERAL VAGOTOMIES *

ROBERT D SLOAN WATTS R WEBB ALBERT L MEENA
AND SAE S LEE

Bilateral cer- —[1]
increase the
long term studies on vagotomized animals have not been feasible since these dogs consistently die within 2 or 3 days following surgery [1] MacCannon and Horvath [2] were able to keep dogs alive by infusion of physiologic saline and glucose given daily for several weeks after the cervical vagotomy Hwang *et al* [3] also using intravenous support during the early postoperative period reported several long term survivals They observed that one of the most common causes of death in these animals was aspiration pneumonitis

Cannon [4] working with cats found that bilateral cervical vagotomy produced cardiospasm or at least abolished the normal receptive relaxation of the cardia However balloon studies by Hwang [3] working with vagotomized dogs demonstrated a constant decrease in tone of the esophagus with incompetence of the cardia as a valve against swallowing or regurgitation

The following experimental procedures were performed to further study the mechanism of the disruption of physiology caused by vagotomy

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METHOD

Bilateral high cervical vagotomies above the right recurrent laryngeal nerve were performed in 14 dogs both for gross survival and for fluoroscopic studies Barium studies were performed in these animals and in normal dogs under very light thiopental anesthesia, which it was felt did not significantly alter the motility of the gastrointestinal tract in the control animals

For these studies a dilute barium mixture was injected through a small plastic catheter which had been inserted into the upper esophagus, and fluoroscopic observations were made for variable lengths of time with the animals in the recumbent or semi erect position

A subsequent series of 10 dogs was subjected to gastroenterostomy or gastroduodenostomy After control gastrointestinal studies some weeks later, bilateral cervical vagotomies were performed and the roentgenograms for esophageal and gastric motility were repeated

RESULTS

Fluoroscopic studies of the injected barium in the normal dog under light thiopental demonstrated vigorous peristaltic waves with resultant effective emptying of the esophageal contents into the stomach Prior to such peristaltic waves, it was noted that the barium would hang at the cardia The esophagus of the normal dog was noted to be much larger in proportion to body size than that of man

All 14 dogs subjected to bilateral cervical vagotomy died within the first 4 days after surgery, even though maintained on intravenous feedings for at least 2 days postoperatively before oral feedings were started Regurgitation and retching were constantly observed, particularly following attempts to eat The postoperative roentgenographic studies on these animals demonstrated a virtual paralysis of the lower esophagus, with marked functional obstruction at the esophagocardiac junction The infrequent peristaltic wave that did occur, however, would carry the barium through the cardia and into the stomach Frequently, the barium was noted to reflux up into the cervical esophagus where it was stopped by the pharyngeal sphincter Postmortem chest x rays demonstated bilateral patchy pneumonitis in each instance Autopsy in each revealed pneumonitis in both lungs plus the usual large esophagus, and rather small shrunken stomach We did not at any time see the picture of complete inversion of the pylorus

In the dogs subjected to gastroenterostomy, subsequent bilateral cervical



Fig 1 Barium swallow (Dog 4) after bilateral cervical vagotomy showing dilated esophagus and contracted cardia



Fig 2 Postmortem chest x ray (Dog 283) showing confluent patchy consolidation which pathologically was an aspiration pneumonia

vagotomy was followed by long term survival in 9 out of 10 dogs Again it was noted that these animals both immediately after the vagotomy and up to 3 months later, showed definite retardation of esophageal motility Nonetheless when the occasional esophageal peristaltic wave did start there was immediate passage of the barium into the stomach In between times there was a definite holdup of the barium at the cardia All dogs with a gastroenterostomy lost weight with some showing ulceration both at the stomach and along the small intestine After vagotomy, these animals usually maintained their weight though none gained significantly

DISCUSSION

These studies would seem to indicate that death in the bilateral vagotomized dog is due to an aspiration pneumonitis As shown by Hwang[3] a large part of the increased emesis after vagotomy is due to sensitization of the upper esophagus and pharynx to the normal emetic reflexes plus a hyperexcitable vomiting center The esophagus after vagotomy shows marked reduction in esophageal motility and delayed emptying This amounts to a functional cardiospasm even though the tonus of the cardia may not be increased Deloyers *et al*[5], working with cats demonstrated definite spasm of the cardia after destruction of the ganglion cells with phenic acid They point out that these injections destroy both the vagal and the sympathetic fibers allowing the circular muscle fibers to reach a state of permanent rest' This can be explained by the experiments of Magnus *et al*,[6, 7] who demonstrated that circular muscle fibers separated from the nervous system reach a state of permanent maximal shortening Vagotomized dogs however, are able to pass sufficient food into the stomach Again vagotomy caused the classic syndrome of gastric atony and pylorospasm or at least failure of the pylorus to relax Thus as the gastroenterostomies protected the dogs following vagotomies vomiting does not seem to be of sufficient magnitude or frequency to cause death in the animal unless reinforced by a gastric reservoir

SUMMARY

Death after bilateral cervical vagotomy in dogs is caused by an aspiration pneumonitis due to regurgitation from a malfunctioning esophagus backed up by an obstructive stomach If the gastric obstruction is relieved the malfunctioning esophagus still has adequate motility and emptying power to maintain viability of the experimental animal

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STUDIES ON THE ROLE OF NERVOUS MECHANISMS IN ANTRAL FUNCTION *

DONALD E WOHLRABE AND WILLIAM D KELLY

The possible effect of vagal innervation on the hormonal function of the gastric antrum has been the subject of considerable investigation and has become a factor in devising operations for peptic ulcer [1,2] The conflicting reports in the literature concerning the role of nervous mechanisms in antral function indicate that these mechanisms are not completely understood [1,3] The following studies were done to determine the effect of vagus denervation of antral pouches upon the hormonal function of the pouches and to study the possibility that an intrinsic nervous mechanism is involved in the release of the antral hormone

METHOD

Seven mongrel dogs were prepared with Heidenhain pouches and with isolated antral pouches drained externally A stainless steel cannula in the Heidenhain pouch permitted the quantitative collection of secretion In 6 of the animals a wide seromuscular bridge was left along the lesser curvature carrying vagal fibers to the isolated antrum In the seventh animal (dog 504) a complete division was done Electrical stimulations of the vagus nerves (subdiaphragmatic) and insulin tests with observation of antral pouch motility confirmed the presence of vagus innervation of the antrum in the first 6 animals and the absence of innervation in dog 504

Following an initial series of studies the antrum was denervated in 5 animals by dividing the seromuscular bridge and the entire lesser curvature mesenteric attachment of the pouch The sixth animal (dog 490) had division of these structures with preservation of the vagus branches to the pouch The antral pouch of dog 504 was again mobilized as a sham procedure at this stage The studies done before denervation of the antrum were repeated Electrical stimulations of the vagus nerves (supradiaphragmatic) and insulin studies demonstrated the presence of vagal innervation of the antrum in dog 490 and its absence in the other animals Dogs 504 and 490 were prepared to serve as controls each having a sham denervation procedure

The daily Heidenhain pouch secretion of each animal was studied before and after the antral denervation procedure Secretions were collected in rubber balloons and free acid determinations were done by titrating with 0 1 N sodium hydroxide using Toepfer s reagent as the indicator The daily output of free acid in milliequivalents was calculated

Beef broth perfusions of the antral pouch were done on each animal during each stage During the perfusions the animals rested comfortably in a modified Pavlov sling The reservoir was kept at a height of 15 cm above the antral pouch stoma and the broth was allowed to run freely into the pouch through a small plastic catheter and out again through the stoma about the catheter A fresh solution of 20% beef extract (USP) was prepared before each study The Heidenhain pouch secretion was collected at 15 minute intervals for a period of 2 hours The free acid output in milliequivalents was determined

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Following the above studies a second series of experiments was performed. Heidenhain pouch responses to broth perfusion of the antral pouch, acetylcholine perfusion of the antral pouch and subcutaneous injection of urecholine were determined. The effects upon these responses of subcutaneous injection of atropine and topical cocainization of the antrum were then studied. The perfusions were performed as described above. An acetylcholine concentration of 1% in water was used. The dose of urecholine employed was 1 mg. Heidenhain pouch secretion was collected every 30 minutes for 2 hours after the injection. The dose of atropine used was 0.6 mg given 20 minutes before the secretory stimulus. Local cocaine effect in the antral pouch during the broth perfusions was obtained by perfusing the pouch with 5% cocaine for 15 minutes before beginning the perfusion and by preparing the broth with a cocaine concentration of 2.5%. A similar method was used with the acetylcholine and urecholine stimuli with the exception that the cocaine concentrations were doubled for these studies.

The animals were maintained on a standard laboratory diet and were kept under standard environmental conditions. Postoperative recovery periods of at least 2 weeks were allowed before studies were begun.

RESULTS

The results of the studies of daily Heindenhain pouch secretion are given in Table 1. The averages given are based upon 25 or more collections from each animal during each stage. The average decrease in secretion following denervation of the antrum in the study group of animals was two times as great as the average decrease in the 2 control animals. This difference between the groups obtains in spite of the fact that 1 animal in the study group differed markedly from the other animals in that it showed an increase in secretion following the denervation procedure.

The results of the beef broth perfusions of the antral pouches are presented in Table 2. The averages given are based upon 5 studies on each animal during each stage. The 15 minute average secretion of free acid was based upon

Table 1 Daily Secretion Before and After Denervation of the Antrum

DOG NUMBER	INNERVATED ANTRUM AVERAGE MEQ FREE ACID PER DAY	DENERVATED ANTRUM AVERAGE MEQ FREE ACID PER DAY	% CHANGE
STUDY GROUP			
417	15.4	16.3	I 6%
721	22.4	2.4	D 85%
757	25.5	9.1	D 64%
786	11.9	5.2	D 48%
795	21.9	5.5	D 75%
Totals	19.4	7.7	D 60%
CONTROLS			
490	28.5	21.9	D 23%
504	6.1	3.9	D 36%
Totals	17.3	12.9	D 29%

I—Increase D—Decrease

the Heidenhain pouch secretion during the second hour of the broth perfusion. It was found that a maximum secretion rate was usually obtained after 45 to 60 minutes of perfusion. No significant difference was found between the average responses of the study group and the control group following antral denervation. Only 1 animal (D-721) had a significant decrease in response (P value .04) following denervation of the antrum. It is of interest that a series of broth perfusions done following subsequent thoracic vagotomy in this animal showed an average increase of 30% in free acid response.

The over-all results of the studies to determine the effect of atropine and antral cocainization upon the responses to the broth, acetylcholine, and urecholine stimuli are given in Table 3. Atropine markedly inhibited the response to each of the 3 stimuli. Cocaine markedly inhibited the response to beef broth perfusion of the antral pouches but had no significant effect upon the response to acetylcholine perfusion or urecholine injection.

Table 2. Broth Perfusions of the Antrum Before and After Denervation

DOG NUMBER	INNERVATED ANTRUM AVERAGE MEQ FREE ACID PER 15 MIN	DENERVATED ANTRUM AVERAGE MEQ FREE ACID PER 15 MIN	% CHANGE
STUDY GROUP			
417	39	21	D 46 2%
721	.63	24	D 62 0%
757	17	.15	D 12 0%
786	.35	34	D 2 9%
795	.58	67	I 15.5%
Totals	.42	32	D 23 8%
CONTROLS			
490	.83	.71	D 14 4%
604	09	.07	D 22 0%
Totals	46	.39	D 15 2%

I—Increase D—Decrease

Table 3. Effects of Atropine and Cocaine Upon Heidenhain Pouch Responses to Broth Perfusion, Acetylcholine Perfusion and Urecholine Injection

STUDY	CONTROL AVERAGE MEQ FREE ACID PER 15 MIN *	WITH ATROPINE AVERAGE MEQ FREE ACID PER 15 MIN *	WITH ATROPINE % CHANGE	WITH COCAINE AVERAGE MEQ FREE ACID PER 15 MIN *	WITH COCAINE % CHANGE
Broth Perfusions	43	03	D 93%	01**	D 98%
Acetylcholine Perfusions	77	15	D 83%	65	D 13%
Urecholine Tests	57	00	D 100%	65	I 11%

I—Increase D—Decrease
* Averages of 8 or more studies ** Average of 5 studies

DISCUSSION

The results obtained from perfusion of the antrum with broth indicate that division of the vagus nerve supply to the antrum *per se* does not significantly alter the ability of the antrum to respond to an appropriate chemical stimulus The broth perfusion method provided a consistent easily controlled and reasonably physiologic stimulus for the release of the antral hormone Since the antral pouches were competely isolated from the gastro intestinal tract other secretory mechanisms and complicating factors such as gastric emptying varying degrees of antral distention and acid inhibition of the antrum were not involved in the responses

The decrease in daily secretion levels after denervation of the antrum suggests that physiologic vagal stimuli are able to activate the antral secretory mechanism and produce a tonic influence on the daily level of acid secretion Oberhelman[1] has suggested that antral peristalsis may be responsible for the release of the antral hormone under these circumstances However no evidence was obtained for release of the antral hormone during the insulin tests done in this study even though high levels of motility were observed in the innervated antrum

The effects of atropine and cocainization of the antrum upon the secretory stimuli studied suggest that an intrinsic nervous mechanism within the antrum plays an important role in the release of the antral hormone during phys iologic chemical stimulation Atropine blocked the secretory effects of the cholinergic stimuli as well as the secretory effect of the broth stimulus Cocaine on the other hand had no significant effect upon the response to acetylcholine or urecholine but it did prevent the response to the broth stimulus This indicates that the site of action of the broth stimulus differs from the site of action of the cholinergic stimuli The most logical explanation seems to be that the latter stimuli act directly upon the cells responsible for the release of the antral hormone while the broth stimulus acts upon some other receptor and the stimulus for release of the hormone is transmitted to the cell by some other mechanism or pathway This mechanism or pathway is probably a nervous one since the transmission of the stimulus is blocked by cocaine

CONCLUSIONS

1 Vagal denervation of the isolated antrum does not significantly alter the response of the antrum to a chemical stimulus (beef broth) as judged by the output of free acid from a Heidenhain pouch

2 Vagal denervation of the isolated antrum results in a moderate decrease in the total daily secretion of free acid from a Heidenhain pouch

3 It is probable that a nervous pathway which is blocked by cocaine transmits the broth stimulus from its site of action to the cell responsible for the release of the antral hormone

4 Cholinergic stimuli appear to act directly upon the cell responsible for release of the hormone and the response is not blocked by cocaine

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THE EFFECT OF CORTISONE AND DIET ON THE ACID SECRETION OF THE HEIDENHAIN POUCH *

R E WIEDERANDERS K L CLASSEN AND W G GOBBEL JR

Controversy still exists concerning the mechanism of stress ulceration Gray[1] combined the now well known fact that the blood level of certain corticoids is increased during stress with the fact that gastrointestinal ulceration may be provoked by stress From this he postulated that pituitary adrenal hyper function is conducive to peptic ulceration His laboratory[2] produced gastric hyperacidity *in man and experimental animals* by injecting ACTH the specific stimulator of these corticoids Zubiran[3] reported that ACTH in creased the gastric acid but not in sufficient quantities to be ulcerogenic however in subsequent experiments from his laboratory[4] this increase was not found Selye[5] stated that hypophysectomized animals were more sus ceptible to gastrointestinal ulceration than intact controls and did not believe that hypersecretion of adrenal corticoids was concerned in the production of these ulcers Using ACTH Sandweiss[6] promoted healing of peptic ulcers in both man and experimental animals

METHOD

Heidenhain pouches were fashioned in mongrel dogs and drained with stainless steel cannulae Each animal was kept in a separate cage and handled by the same individual throughout the experiment All pouch secretions were collected at 24 hour intervals At the time of collection the collecting balloons were carefully checked to be sure that no leaks were present The volume of each specimen and the amount of free and combined hydrochloric acid were measured

RESULTS

Effect of Cortisone Acetate The dietary intake of these 8 dogs was kept constant throughout this experiment Pouch secretions were collected for 30 days on each dog The first 10 days served as the control period During the second 10 days 300 mg of cortisone acetate were given intramuscularly each morning The third 10 days served as the second control period (Fig 1) Five dogs increased their free acid output by an average of 63% 3 decreased by an average of 16% This was an increase of 27% for the group

Effect of Diet Since the antrum with its hormonal function was left intact it seemed likely that these animals would respond to variations in the amount of food eaten An attempt was made to determine specifically the effect of this mechanism Three different arbitrary amounts of dog food were chosen and are here designated as diets The low diet contained 240 gm standard diet—540 gm and high diet—1500 gm of food Three dogs were placed on the standard diet for 10 days high diet for 10 days and returned to the standard diet for the final 10 days (Fig 2) These increased their free acid output by an average of 293% Four animals were given low diet—high diet—low diet feedings for the same number of days These increased their outputs by an average of 439%

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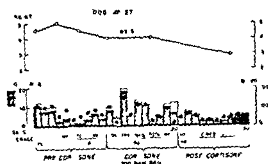


Fig 1 Free and total hydrochloric acid response in a dog typical of this group receiving 300 mg of cortisone while on a rigidly controlled diet

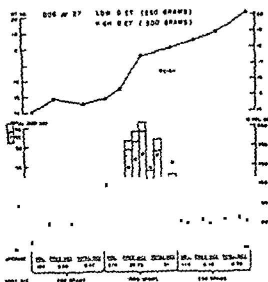


Fig 2 Showing the profound rise in free hydrochloric acid produced by increasing the diet from 250 to 1500 gm Notice the immediate return when the high diet is stopped

Effect of Adrenalectomy. Adrenalectomy was carried out on 2 dogs These animals were maintained on a rigidly controlled standard diet throughout the experiment Each was maintained in good health for at least 3 months In each dog the daily maintenance dose of cortisone acetate was increased each 10 days For the first 10 day period each dog received 10 mg /day Each 10 days thereafter the dose was increased to 20, 40 80 and finally 300 mg cortisone/day (Fig 3) There was a decrease in the average daily free acid production of 18 and 75% respectively in the 2 dogs when the cortisone dosage was increased from 10 to 300 mg /day

DISCUSSION

Three hundred milligrams of cortisone acetate, which represents a large amount of this drug for a dog, was chosen because others[2, 3, 4] have reported conflicting results from administering smaller doses During the time this quantity of cortisone was given, an average increase in the free acid output of 27% was found This increase was not uniform for these animals and 3 of the dogs showed a decreased output during this period

In contradistinction to these results were those obtained from increasing the amount of food the animals ingested The increase in free acid was marked reproducible and roughly proportional to the dietary increase Increases in acid production up to 597% were readily produced, probably through the antral and intestinal mechanisms

Removal of the adrenal glands should abolish any effect they may have to alter the action of exogenous cortisone In both animals so treated, a drop in acid output occurred as the cortisone was increased

The increase during cortisone administration does not appear significant when the usual daily variations in acid output and the great increase produced by diet are considered

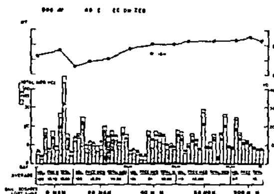


Fig 3 Acid response in an adrenalectomized dog receiving progressive increments of cortisone while on a rigidly controlled diet

CONCLUSIONS

Cortisone acetate does not produce a significant hyperacidity in the Heidenhain pouch in the dog Since variations in diet are reflected in the gastric hydrochloric acid output the hyperchlorhydria reported by other observers may be due to dietary indiscretion on the part of the animals

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GASTRIC PEPSIN SECRETION IN PATIENTS WITH CRANIAL INJURIES WITH RELATION TO THE DEVELOPMENT OF ACUTE PEPTIC ULCER *

CLIFFORD M PHIBBS AND LLOYD D MACLEAN

The development of an acute peptic ulcer as a complication of major surgery stress or trauma has received much interest and attention since the report of

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Cushing[1] in 1932 suggested a relationship between the interbrain and the development of acute peptic ulceration The reviews of Davis,[2] Harkins[3] Gregg[4] and others have suggested a relationship between acid peptic ulceration and major surgery, stress, trauma, or intracranial lesions

The present study was designed to evaluate the alterations in gastric secretions in patients subjected to major trauma or stress such as head injuries multiple fractures, and emergency surgery, as well as in control patients and ulcer patients The questions we wished to answer were 1) is one type of injury more likely to lead to acid peptic ulcer, 2) is gastric secretion elevated in patients who develop stress ulcers or is an alteration in the protective capacity of the gastrointestinal mucosa a more likely cause of this form of ulceration? One advantage of this study was that in several instances we were able to follow the patient and his gastric secretions until development of an acute peptic ulcer and not infrequently until death and the subsequent postmortem

METHOD

The studies were made on 12 hour gastric aspirates, nocturnal whenever possible, obtained by using a 9 hole #16 nasogastric tube on constant suction No liquids were used to irrigate the tube Specimens for pepsin were collected on ice and frozen until the determinations were made Each specimen was analyzed for appearance, volume, free and total acid, pH and pepsin Pepsin determinations were done by the method of Anson[5] The pH was determined using a Cambridge microelectrode pH meter Two successive 12 hour specimens were obtained shortly after admission on patients with cranial injuries and with multiple fractures with followup specimens approximately every other day until recovery or death On surgical patients a 12 hour specimen was obtained the night before surgery and the postoperative specimens were obtained in the same manner as for patients with head injury

RESULTS

The results on 78 patients reported in terms of 12 hour specimens, are summarized in Table 1 Pepsin concentration and total 12 hour output of pepsin were found the most valuable indices of gastric secretion in this study and will be stressed in outlining the results and in the discussion The volume, pH and pepsin (0 62 mg /cc) in the control patients are similar to that reported by other workers[6] Levin[7] has reported a wide individual variation of gastric secretion from hour to hour and from night to night but a continuous 12 hour gastric aspiration was the most desirable

Gastric secretion in patients with cancer of the stomach was markedly depressed Pepsin concentration was 0 28mg /cc In patients who underwent routine, elective uncomplicated surgery, gastric secretion was slightly depressed in the early postoperative period Pepsin concentration was 0 61 mg /cc

Pepsin secretion was slightly increased in patients with gastric ulcer (0 78 mg /cc) and markedly increased in patients with duodenal ulcer (1 31 mg /cc) Patients undergoing emergency surgery who were acutely ill with such problems as bowel obstruction, gangrenous appendicitis, acute cholecystitis and empyema of the gallbladder produced a gastric secretion of high pepsin concentration (1 11 mg /cc)

The highest pepsin concentration and the highest total output were found

Table 1 Results of Gastric Aspiration in 78 Patients Studied

	NUMBER	VOLUME	FREE ACID	TOTAL ACID	pH	PEPSIN MG/CC	TOTAL PEPSIN PER 12 HOURS IN MG
Controls	10	342 (10 548)	9 5 (0 16)	39 6 (5 50)	3 9 (2 7 7 1)	0 62 (0 2 1 1)	205 (88 450)
Elective surgery	22	174 (5 145)	4.5 (0 23)	21.2 (1 73)	4 5 (2 0 8 2)	0 61 (30 1 7)	119.6 (18 383 6)
Gastric ulcer	7	435 (119 1285)	15 9 (0 66)	49 2 (11 100)	3 36 (1 28 6 13)	0 78 (37 1 21)	389 4 (47 6 1554)
Multiple fractures	8	336 (29 1100)	7 3 (0 25)	41 7 (15 68)	3 43 (1 77 5 7)	1.22 (45 2 2)	513 0 (19 3 2420)
Duodenal ulcer	6	603 (25 1570)	7 8 (0 60)	31 8 (18 84)	3 29 (1 62 4 28)	1 31 (29 1 8)	969 0 (32 5 2512)
Cancer of stomach	4	186 (90 280)	0 0	17 8 (7 36)	4 64 (2 73 6 49)	0 28 (0 2 41)	51 7 (30 6 952)
Emergency surgery	9	463 (16 1480)	10 5 (0 49)	31 2 (0 77)	3 84 (1 63 8 18)	1 44 (0 2 3 6)	736 6 (3 2 2960)
Head injuries	12	600 (86 1030)	16 5 (0 59)	68 9 (34 117)	2 95 (1.27 4 3)	1 64 (51 4 0)	1245.5 (86 3196)

Data on gastric secretory volume free gastric acid total gastric acid pH pepsin in mg/cc, and total 12 hour pepsin in all patients studied

in patients with cranial injury (1 64 mg/cc pepsin or 1245 mg/12 hours)

A total of 8 of the 78 patients studied developed either upper gastro intestinal hemorrhage in the postoperative or postinjury period, or had an acid peptic ulcer or erosions demonstrated at postmortem (Table 2) The latter were an antemortem development in each instance Five patients had cranial injury, 2 emergency surgery, and 1 a fractured hip All 8 patients secreted a gastric juice with pepsin concentration which exceeded the average for duodenal ulcer patients

DISCUSSION

In a study of 78 patients it was found that cranial injury, multiple frac tures, and emergency surgery in acutely ill patients caused an increased volume and acidity of gastric secretion and a markedly increased pepsin out put The highest rates of pepsin production were in the patients with the more extensive injuries Furthermore, those patients with pepsin secretion which exceeded that found in patients with duodenal ulcer were exceedingly likely to develop upper gastrointestinal hemorrhage from ulceration of the stomach or duodenum The pepsin concentration in these patients was found to remain elevated as long as 1 week following the injury The gastric secre tions of many patients who did not develop hemorrhage but had an elevated pepsin output for 24 to 48 hours, returned to normal before 1 week had elapsed

An elevated pepsin output which persists in a severely injured patient (par ticularly cranial injury) we believe to be of prognostic importance The use of prophylactic measures to avoid gastrointestinal hemorrhage is indicated Studies utilizing total body cooling are in progress Local cooling of the stomach as suggested by Wangensteen *et al*[8] may be more effective This in vestigation supports the thesis that increased gastric secretion, particularly pepsin, is responsible for acid peptic ulcers seen following stressful situations

Table 2 Pepsin Secretion of Patients Who Developed Acid peptic Ulcers or Gastrointestinal Hemorrhage After Trauma

	FIRST SPECIMEN		THIRD DAY		ONE WEEK	
	MG /CC.	12 HOUR TOTAL	MG /CC.	12 HOUR TOTAL	MG /CC.	12 HOUR TOTAL
Extradural hematoma	4 0	1016	3 8	1710	1 7	1561
Fractured hip	2 2	2120				
Severe head injury	1 3	611	2 3	1081	3 3	571
Cerebral concussion	2 74	2137			2 77	1468
Cerebral concussion	1 7	3196	1.2	1140	1 0	730
Cerebral hemorrhage	2 12	191	2 39	302	1 3	338
Emergency surgery	2 0	2960	4 6	2024	3.2	976
Emergency surgery	1 1	363	1 6	1800	1 6	557

Gastric pepsin secretion in 8 patients who developed upper gastrointestinal hemorrhage

SUMMARY AND CONCLUSIONS

1 Gastric secretion with particular reference to pepsin has been studied in 78 patients by use of the 12 hour continuous suction technique

2 The investigation was carried out in normal individuals and in the following groups of patients those with gastric cancer gastric ulcer, duodenal ulcer multiple fractures cranial injuries and in patients subjected to surgery both elective and emergency

3 Markedly increased pepsin secretions both concentration and total 12 hour output were found in patients with cranial injury those undergoing emergency surgery and in patients with multiple fractures

4 Eight patients developed upper gastrointestinal hemorrhage with without a demonstrated ulcer Five had severe cranial injury preceding had persistently elevated pepsin secretion which exceeded that found patients with duodenal ulcer

5 The measurement of gastric pepsin output in severely injured patients suggested to determine those patients likely to develop acute acid peptic ulcer Suggestion for prophylactic treatment is made

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THE SECRETORY RESPONSE OF THE DENERVATED GASTRIC POUCH TO PROLONGED MASSIVE CORTISONE ADMINISTRATION *

LAWRENCE S McGEE JR JOHN BLACKBURN
EDWARD M LANCE AND H WILLIAM SCOTT JR

The apparent activation of peptic ulcers in patients receiving adrenal steroids has been studied experimentally for some time The work of Dragstedt[1] Gray[2] French[3] Sandweiss[4] and others has produced conflicting data and opinions

Critical analysis of previous experimental studies of cortisone s effect o gastric secretion reveals the following deficiencies 1) many experiments have been acute in nature i e of six to twenty four hours duration 2) results frequently have been expressed in concentration of free acid rather than total free acid output ignoring the generally accepted fact that the latter is of prime importance in the genesis of peptic ulcer 3) Levine tube sampling with the disadvantages of duodenal reflux and partial aspiration of gastric con tents has been used by some 4) data have been obtained from anesthetized animals 5) monitoring of serum steroids and electrolytes has been neglected in animal experiments 6) the response to massive doses of steroids has not been tested This experiment was designed to avoid the above criticisms in a relatively large number of animals

METHOD

Healthy adult mongrel dogs were subjected to the creation of a denervated gastric pouch (the Heidenhain pouch) An indwelling stainless steel cannula attached to a large thick rubber balloon was used to collect secretions Twenty four hour collections were analyzed for volume free and total acid pH sodium potassium and chloride Weekly determinations of serum sodium potassium chloride protein and 17 hydroxycorticoids were done The dogs were weighed weekly Throughout the experiment they were given a uniform quantity of Purina Dog Chow and water *ad lib* After an adequate control period intramuscular cortisone acetate in the amount of 300 mg daily was given for 2 weeks

A second group of animals similarly prepared was subjected to removal of the gastric antrum and the study repeated

A third group with the antrum intact received daily local instillation of 300 mg of cortisone acetate † into the Heidenhain pouch

For purposes of analysis the collections were grouped as follows a) the control period b) the period of cortisone administration, c) the first 2 weeks after stopping cortisone (Recontrol #1) and d) the second 2 weeks after cortisone (Recontrol #2)

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† Supplied by Dr Elmer Alpert of Merck Sharpe & Dohme

* From the S R Light Laboratory for Surgical Research and the Department of Surgery Vanderbilt University School of Medicine Nashville With the technical assistance of Rachel K Younger

RESULTS

Antrum Intact In all 6 animals with antrum intact there was a significant rise in 24 hour free acid during the period of intramuscular cortisone administration The average 24 hour secretion of free acid (expressed in mEq) increased during the cortisone period over control values by 35 51, 69 80 82 and 185% In 5 of the animals the average 24 hour secretion returned to control levels during the second 2 week period after cortisone (Recontrol #2) It was in this period that 17 hydroxycorticoids returned to near normal levels The sixth dog died after only 11 days of cortisone administration of a perforated pouch ulcer

The observed increase in free acid secretion was due primarily to an increase in volume as the pH decreased little or none Electrolyte studies of all gastric secretions revealed a consistent drop in sodium but no significant change in potassium or chloride during cortisone administration

While on cortisone each animal s serum potassium dropped sodium showed a slight tendency to rise and serum chlorides for the most part remained normal However in 1 dog which had a 185% increase in acid secretion serum chlorides dropped to 70 mEq /L Blood steroid values rose to high levels in each instance and in several cases had not returned completely to normal 4 to 6 weeks after stopping therapy Body weight did not change significantly

Antrum Out Four of the 5 dogs with antrum removed failed to show any significant increase in twenty four hour acid secretion while on cortisone A fifth animal showed a pronounced rise As the volume and acid secretion of this dog s pouch were much higher during the control period than the average it is suspected that a portion of its antrum was not excised In this group there was little change in values of electrolytes of pouch secretions Serum electrolytes and steroids showed identical patterns to those previously described

Local Instillation of Cortisone into Pouch Two dogs which received local daily instillations of cortisone into the Heidenhain pouch showed no significant change in acid output

Figure 1 shows the daily free acid values of representative animals Table I summarizes the important data of all animals

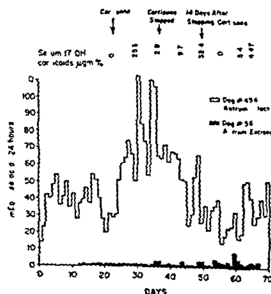


Fig 1

Table 1 The Response of the Canine Heidenhain Pouch to 300 Mg. Cortisone Daily

AVERAGES OF 24 HOUR VALUES WITH THE ANTRUM INTACT

	CONTROL				CORTISONE				RECONTROL #1				RECONTROL #2			
	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID
Dog 353	31	328	1 6	35	11	480	1 5	63	—	—	—	—	—	—	—	—
Dog 410	33	249	1 7	24	11	562	1 7	71	11	438	1 8	60	27	348	1 7	43
Dog 494	23	325	1 8	38	14	475	1 7	69	13	459	1 7	57	21	261	1 4	31
Dog 478	31	275	1 6	32	14	408	1 6	54	14	346	1 7	51	5	161	1 8	20
Dog 515	22	375	1 8	45	14	428	1 7	61	—	—	—	—	19	352	—	38
Dog 523	21	401	1 9	33	14	414	1 6	50	12	276	1 8	29	22	394	—	41

AVERAGES OF 24 HOUR VALUES WITH THE ANTRUM EXCISED

	CONTROL				CORTISONE				RECONTROL #1				RECONTROL #2			
	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID	DAYS	VOL	pH	mEq FREE ACID
Dog 515	9	43	1 6	2 6	13	36	1 5	2 8	10	49	1 6	5 6	13	55	1 6	2 8
Dog 530	8	26	—	4	6	25	—	8	6	21	—	1 0	DEAD			
Dog 511	6	61	1 0	5 5	14	52	1 2	5 8	DEAD				—	—	—	—
Dog 554	10	74	1 4	4 6	14	130	1 5	17 4	13	172	1 2	21 0	7	203	1 4	25 0
Dog 567	10	17	3 8	2	14	22	1 9	1 4	14	24	1 9	1 6	15	38	1 6	3 0

CONCLUSIONS

Parenteral cortisone in large doses will produce a significant rise in the twenty four hour acid output of a denervated gastric pouch. In animals on similar doses of cortisone but with the antrum excised there is no increase in acid secretion

The mechanisms underlying the rise in acid in the first group are poorly understood Certainly the antrum appears to be of prime importance in mediating the response to cortisone stimulation Studies of overnight fasting secretions in animals with an intact antrum are now in progress

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THE EFFECT OF PANCREATECTOMY ON THE INCIDENCE OF GASTRIC ULCERATION IN THE SHAY RAT*

RENE B MENGUY AND MERLIN K DUVAL, JR

In 1931 Elman and Hartmann found that diversion of pancreatic juice from the duodenum resulted in duodenal ulcers in all of the animals that they studied This was confirmed later by Dragstedt who reported that external drainage of pancreatic juice was followed by peptic ulceration of the duodenum in 100% of his dogs These findings were attributed to removal of the acid buffering mechanism of pancreatic secretions However, further studies by Dragstedt[1] revealed that in the depancreatized dog the incidence of peptic ulcer was only 3% Since the acid buffering capacity of the duodenal contents should be decreased by pancreatectomy to the same extent as by diversion of pancreatic secretions one is tempted to invoke a decreased output of gastric acid in the depancreatized animal Attention has been refocused on this problem by the now classical observations of Zollinger and Ellison[2] Their original hypothesis was that the "ulcerogenic factor" in the blood of patients with non beta islet cell tumors of the pancreas was the hyperglycemic glycogenolytic factor or glucagon However, several experimental studies[3, 4] have shown that, instead of acting as a stimulus to gastric secretion, glucagon seems to inhibit such secretion Regarding this problem of the relationship between some internal factor of the ... ques tions have been rai ... and

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Dragstedt[5] who reported that, in a small series of dogs the output of HCl from Heidenhain pouches was increased after pancreatectomy

Our investigations were undertaken as a pilot project in an attempt to establish further experimental evidence for the existence of a stimulatory hormone of pancreatic origin Our test preparation was the pyloric ligated or Shay rat Ligation of the pylorus in a fasting rat results in rapid development of multiple gastric ulcers Our initial premise was that if the pancreas in the rat elaborated a hormone with a stimulatory action on gastric secretion, simultaneous pancreatectomy in the Shay preparation would provide some protection against gastric ulceration

METHOD

Male albino rats of the Holtzman strain weighing from 300 to 350 gm were used The animals were fasted for 72 hours prior to the experiments During this time they were kept in individual metabolic cages with wide mesh wire bottoms and permitted water *ad lib* The animals were divided into two groups by random selection In one group, a pyloric ligation according to the method of Shay was carried out In the other, simultaneous pyloric ligation and pancreatectomy were performed An estimated 90% of the pancreas was removed At the end of 24 hours blood was withdrawn for blood sugar analysis and the animals were killed The gastric contents were carefully measured for volume and analyzed individually for concentration of HCl The emptied stomach was then divided along the greater curvature pinned on a board and the ulcers in the rumen were counted with dissecting lenses The average of 2 counts by 2 observers was recorded Results are given in Table 1 The number of rumenal ulcers in the depancreatized rats was appreciably smaller than in the controls Ulcers, when present, were small, discrete, and the gastric mucosa did not have the shaggy, edematous appearance characteristic of the control animals The volume of gastric contents in the depancreatized animals was also significantly lower than in the test group The contents were usually clear whereas in the control animals hemorrhage was always present On account of this gastric hemorrhage, analyses of gastric contents for HCl concentration were invalid and are not presented It is notoriously difficult to produce diabetes in the adult rat by pancreatectomy None of the depancreatized rats had a blood sugar in the diabetic range 24 hours after pancreatectomy In effect, this facilitated the interpretation of our data

To rule out the possibility of our results being due to a more extensive operative trauma in the pancreatectomy group, another control experiment was done In 5 rats simultaneously with pyloric ligation, the spleen was removed and the pancreas was mobilized from the greater curvature of the stomach as in pancreatectomy All 5 rats had the high ulcer rate characteristic of the Shay preparation

The decreased gastric volumes and low ulcer counts in the depancreatized rats could have been due simply to removal of pancreatic secretions from the duodenum In order to rule out this possibility, we performed a series of experiments in which the drainage of pancreatic juice into the intestine was interrupted and the pancreas left *in situ* Initially, we ran into some difficulty in attempting selective interruption of the flow of pancreatic secretions In the rat the pancreatic ducts are multiple and drain into the common bile duct

Interruption of flow of pancreatic juice into the intestine is best accomplished by ligation of the common bile duct at its junction with the duodenum. However, this also prevents bile from reaching the duodenum and we found, thus confirming some of Madden's observations, that biliary obstruction alone decreased gastric secretion and protected against gastric ulceration in the Shay preparation. This difficulty was circumvented by cannulating the common bile duct in the hilus of the liver with a short length of #50 polyethylene tubing and inserting the distal end of the tubing into the duodenum. Control experiments showed that rats with this type of bile shunt had the usual number of ulcers in the gastric rumen 24 hours after pyloric ligation. We were then able to compare this control series with a series of rats in which we ligated the distal end of the common bile duct (in addition to creating a bile shunt and ligating the pylorus), thus interrupting only the flow of pancreatic secretions to the duodenum. The gastric volumes and ulcer rates in this series were high (Table 2).

DISCUSSION

One may raise the objection that the gastric ulcerations in the Shay rat preparation do not resemble chronic peptic ulcer in man. Nevertheless, both processes appear to be related to acid pepsin erosion. In this respect the Shay rat preparation is a useful screening tool prior to more specific investigative methods. At present our data show that in the depancreatized rat pyloric ligation resulted in smaller gastric volumes and lower ulcer rates than in the controls. On the other hand, suppression of the flow of pancreatic secretions into the duodenum had no such protective effect, thus indicating that the

Table 1

	18 CONTROL RATS	13 DEPANCREATIZED RATS
Weight †	325 gm	343 gm
Ulcers in rumen †	52	6
Perforations (%)	33	0
Gastric content †	16.3 cc.	7.3 cc
Blood sugar †	212 mg %	116 mg %

† average

Table 2

	9 CONTROL RATS (PYLORIC LIGATION AND BILE SHUNT)	15 PANCREATIC DUCT LIGATED RATS
Weight	331 gm	364 gm
Ulcers in rumen	71	57
Perforation (%)	1	0
Gastric content	16 cc.	14.5 cc

protective mechanism of pancreatectomy was not due to removal of pancreatic secretions from the duodenum Although these data lend support to the concept of a stimulatory hormone of pancreatic origin the evidence is far from conclusive For instance it is conceivable that the effects of pancreatectomy on gastric secretion are due to a disturbance of endocrine relationships between the pancreas the hypophysis and the adrenals rather than to the suppression of a specific stimulatory hormone

SUMMARY

Shay rat preparations with and without simultaneous random removal of the pancreas were carried out None of the depancreatized rats became diabetic during the period of observation Pancreatectomy provided almost complete protection against gastric ulceration in the Shay rat Control experiments showed that this protection was not due to removal of pancreatic secretions from the duodenum

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FULL THICKNESS GASTRIC MUCOSAL EXCISION IN CATS WITH PROLONGED SURVIVAL RESISTANCE TO HISTAMINE INDUCED ULCERATION *

F R C JOHNSTONE

Because we have no certain knowledge of the etiology of peptic ulceration modern surgical therapeutic procedures devised to deal with ulceration are generally based on some method of reducing acid secretion Assuming acid secretion to be the function of the parietal cell these measures have commonly involved resection of the body and antrum of the stomach which for technical reasons has also included the submucosa muscularis and serosa Attempts to remove merely the mucosa have not been successful If the whole thickness is removed regeneration does not occur but healing of the denuded area is by scar tissue [1] It has been shown possible to graft the raw bed [2, 3] but the results were none too satisfactory On the other hand if only the superficial portion of the mucosa is removed rapid regeneration is the rule [4] Attempts have been made to destroy the mucosa by radiation both internally

* From the Department of Surgery University of British Columbia Vancouver B C Supported in part by the Leon and Thea Koerner Foundation With the assistance of A M Read B.A.Sc

and externally, but total destruction is followed by scarring, incomplete destruction by regeneration[5]

The rather simple procedure reported here has not previously been done. The mucosa is excised and lumen continuity restored by anastomosing the two cut edges of the mucosa, leaving the serosa and muscularis as a redundant pouch devoid of mucosa (See Fig. 1 to 5) Varying amounts of the total thickness of the mucosa can be removed from various regions of the stomach. In this way the gastric secretion can be reduced without interference with the serosal surface, division of the underlying vagus nerves, or transection of muscle with possible interference to continuity of peristaltic contraction. It also leaves the cardiac and pyloric orifices intact. The reduction in secretion has been tested by observing the response to attempts to induce ulceration by histamine.

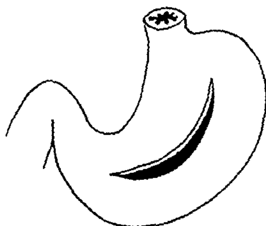


Fig 1 Incision in anterior wall of stomach

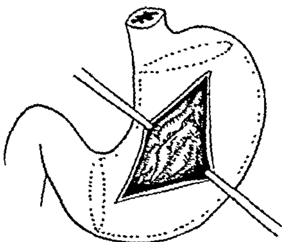


Fig 2 Extent of mucosa removed

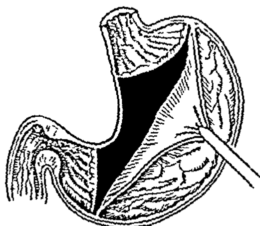


Fig 3 Separation of mucosa from underlying muscularis

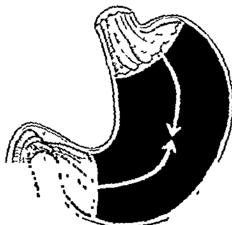


Fig 4 Approximation of mucosa necessary.

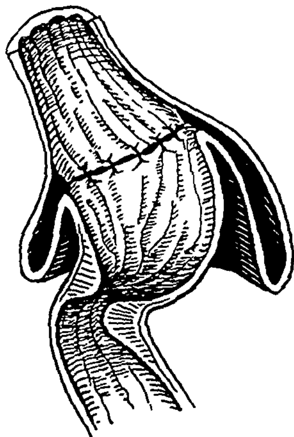


Fig 5 Mucosal anatomosis completed showing redundant seromuscular pouch

METHODS

Cats were employed and were starved for 24 hours prior to operation Under nembutal anesthesia and sterile conditions through a midline abdominal incision, the stomach was delivered With diathermy the anterior wall of the stomach was incised in its long axis, midway between the curvatures The whole thickness of mucosa was then excised At the time of operation the length and situation of the mucosa remaining was measured Measurement was taken from either the cardia or pylorus to the cut edge of the mucosa in the long axis of the stomach, midway on the anterior wall This distance was checked at autopsy The autopsy measurements are the ones given Bleeding from the raw bed tended to stop by the contraction of the muscularis and any continuing bleeding was dealt with by coagulation and occasionally undersewing of the vessels No ligatures were placed on the vessels externally The cut mucosal edges were apposed with 4 0 chromic catgut sutures and the incision in serosa and muscularis anteriorly closed with 4 0 black silk The abdomen was closed in layers The cats received subcutaneous saline, and penicillin 100,000 units i m for 2 days postoperatively and were then started on milk and water by mouth and by the seventh day were on a normal diet of milk and canned cat food Approximately 2 months after operation they were subjected to induction of ulceration by histamine in beeswax [6] Initially 20 mg of histamine were given daily for 6 days in each week If the animals maintained their weight and wellbeing the dose was increased until up to 75 mg daily were given In the later experiments, dosage was 60 mg daily from the start Each group was given histamine until all the control cats had died The survivors were then sacrificed and their gastrointestinal

tract examined for ulceration One control cat was sacrificed when it was in extremis and was found to have a deep gastric ulcer penetrating into liver.

Thirty cats were subjected to mucosal excision. Thirteen cats failed to survive the postoperative period. Of these, 3 did not recover from the anesthetic, 2 bled into the seromuscular pouch and in 7 the mucosal suture line separated with gross sepsis involving the pouch, which was followed by peritonitis in 3 One died of inanition due to partial separation of the suture line with subsequent scarring. Seventeen cats survived for periods from 2 to 11 months One died of empyema in the third month. The operative site was healthy. Five cats gained weight postoperatively, 6 maintained their weight and 6 lost weight. Two additional cats were subjected to retrocolic antiperistaltic partial gastrectomy. Both lost weight. Eight of the mucosal resection cats and the 2 partial gastrectomy cats were subjected to histamine injections.

Four cats with only 2 cm. of mucosa remaining at both the cardia and at the pyloric antrum survived without ulceration. Two cats developed ulceration One cat, which died from a perforated peptic ulcer at the lower end of the esophagus, had 6 cm of mucosa remaining at both cardia and antrum Another cat survived the histamine injection, but was found to have a penetrating ulcer in the duodenum In this cat 6 cm of cardiac mucosa and 3 cm. of antral mucosa remained In 2 cats the mucosa of the lesser curve was left, repair being effected by suture of the mucosa in the long axis of the stomach on the greater curve side. Both died from perforated duodenal ulcers In addition to the 2 cm rim of mucosa at the cardia and pylorus, a considerable amount of lesser curve mucosa was needed to form a tube of adequate lumen Both the cats which had partial gastrectomies died, one from a hemorrhage from a stomal ulcer, one from a perforated stomal ulcer One had a 2 cm, the other a 4 cm cardiac rim of mucosa remaining Of the 7 control cats, 2 had sham operations. The mucosa of the stomach was incised, the incision being repaired in the same way as the cats with the mucosal excision. Both of these died, one from a bleeding duodenal ulcer, the other from a perforated duodenal ulcer The site of the mucosal incision did not show ulceration Two of the others died from perforated duodenal ulcers Two died from bleeding duodenal ulcers One was sacrificed when moribund and was found to have a deep gastric ulcer, penetrating the liver.

DISCUSSION

As part of these investigations, the situation of the parietal cells in the cat's stomach has been mapped There are relatively few parietal cells at the cardia, but they quickly become numerous, reaching a maximum in the fundus and body, to disappear almost wholly from the distal 4 cm of the antrum Thus, those animals in which the number of parietal cells has been reduced by an adequate excision of the mucosa, successfully resist histamine induced ulceration When, however, too great a portion of the proximal gastric mucosa, and thus too many parietal cells remain, ulceration occurs This is in accordance with the view that histamine in beeswax produces its effect by prolonged increase in the secretion of hydrochloric acid Other mucosal excisions, not reported here suggest that the ulceration occurring at the stoma in the cats subjected to partial gastrectomy is due to the absence of the antrum

It was not possible in these animals to carry out gastric analysis, but contamination by saliva and intestinal juice would probably invalidate such

estimations Technically, a greater survival *rate from operation* is possible by preliminary ligation of the gastric *vessels supplying the* portion of the stomach to be denuded This was purposely not done in order to reduce the number of extraneous factors *The restoration of the* remaining mucosa *to an apparently* anatomical normal is shown in Figure 6 The mucosa had *been excised* 6 months previously, in extent 2 cm from the cardia to 4 cm *from the pylorus. Despite* the loss of gastric mucosal surface, the majority of the cats remained well, some in fact improving their weights, and one cat even produced a normal litter of *kittens, which she reared.*



Fig 6 Stomach 6 months following excision of gastric mucosa with restoration of lumen continuity by mucosal anastomosis

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TOTAL EXCISION OF ACID SECRETING GASTRIC MUCOSA IN THE DOG *

H T G WILLIAMS W PISESKY AND WALTER C MACKENZIE

Although partial gastrectomy has given satisfaction to a large number of patients with peptic ulceration there is an increasing awareness of the frequent unpleasant and even disabling symptoms that may follow this operation

Not only is the size of the stomach reduced but loss of the pylorus makes it unable to retain any food Radiological studies have shown that after gastrectomy food passes into the jejunum with little evidence of any storage in the stomach [1]

The rapid passage of hypertonic material into the jejunum causes a great outpouring of intestinal juice This results in both hyperperistalsis and reduced circulating blood volume which is believed to be the basis of the dumping syndrome [2, 3] The intestinal hurry results in inefficient digestion and absorption so that in time nutritional deficiencies develop [4, 5] Once these complications appear their treatment is usually unsatisfactory

The ideal operation should reduce or eliminate the acid and pepsin secretion of the stomach and leave its storage function intact The logical method would be a direct attack on the acid secreting mucosa

Longmire and his associates [6-8] have suggested 3 possible ways of doing this *Excision* Full thickness excision of the mucosa leaving the area to granulate and epithelialize produces gross scarring and a stomach which has very thick rigid and fibrotic walls lined with columnar epithelium *Replacement with non-acid secreting mucosa* Partial thickness excision and the application of jejunal grafts was possible on a small scale but not practicable when applied to the whole of the acid forming area *Alteration in the cellular structure of the gastric mucosa* It was shown that a partial thickness excision of the gastric mucosa was followed by a growth of nonspecific columnar cells which lined the stomach This produced a significant hypochlorhydria and drop in peptic activity Within 12 months parietal and peptic cells had reappeared and the acid level returned to 75% of normal

Applying Longmire's suggestion of excision the following experimental operation has been developed

METHOD

The stomach is freed along the greater curvature from the proximal antrum to the esophagus Atraumatic clamps are then placed across the proximal antrum ¼ from the junction with the body and across the gastroesophageal junction A Blalock clamp is used to control the left gastric artery one of its jaws passing through the same hole in the dorsal mesentery as the gastroesophageal clamp In this way the blood supply is completely controlled (Fig 1 1)

The mucosa is exposed by an incision along the greater curvature (Fig 1 2) There is no bleeding except for transient loss from filled veins The inside of the stomach is carefully swabbed out The wound is packed off around the opened stomach

* From McEachern Cancer Research Laboratory and Department of Surgery University of Alberta Edmonton

Excision of the mucosa is now started An assistant holds the mucosa taut with a series of Duval s tissue forceps It is then a simple matter to peel off the seromuscular coat from the mucosa stroking the plane of separation with a scalpel, and cutting down on to the taut mucosa (Fig 1 3)

The stripping is completed on the anterior and posterior walls so that the separations meet at the lesser curvature (Fig 1 4) The bridge of mucosa is then divided to leave funnel shaped mucosa leading up towards the esophagus and down towards the antrum (Fig 1 5) Each funnel shaped mucosal area is stripped to a point ½" from the controlling clamps The upper and lower mucosal funnels are excised so that no freed mucosa remains this avoids the risk of necrosis when the anastomosis is made

Removal of the 2 funnel shaped portions of mucosa leaves 2 mucosal edges one ½" from the gastroesophageal junction and the other in the proximal antrum (Fig 1 6) Figure 1 10 shows this in section By this time the exposed seromuscular layer has contracted down to at least ¼ of its original size On its surface it presents several tiny elongated holes, especially in the region of the lesser curvature These are the divided vessels which passed through the muscle layers to anastomose in the submucosa Each is picked up with fine artery forceps and tied off

A direct end to end anastomosis between the 2 mucosal edges is performed with 000 chromic catgut, inverting the anterior layer (Figs 1 7, 1 8) Each bite of the needle should pass through the mucosa, submucosa and just into the muscle layer so that it will hold securely and provide hemostasis (Fig 1 11)

At this stage the anastomosis is surrounded by the bare seromuscular coat the edge of which is held up by tissue forceps (Fig 1 8) The clamps controlling the blood supply to the stomach are then removed There is free oozing from the raw surface of the seromuscular layer, which stops after packing with dry gauze for about 5 minutes This layer retains a good colour *With a continuous inverting mattress suture the seromuscular layer is closed* over the anastomosis leaving very little dead space (Fig 1 9) Figure 1 11 shows the stomach in section at the end of the operation

RESULTS

The operation was performed on 15 dogs They withstood the operation well and were back on a normal diet within a week

Three of the dogs were killed at intervals of 1, 5, and 12 weeks after the operation to see how healing progressed The space between the seromuscular coat and the anastomosis was filled with clot which was completely absorbed by the fifth week At this time the seromuscular layer had contracted down to form a thickened fold around the anastomosis (Fig 1 12) By the twelfth week there was little evidence of this thickening and the stomach was supple and mobile There was never any suspicion of fibrous stenosis at the anastomosis in any of the 15 dogs

A laparotomy was carried out on 2 of the dogs 10 weeks after their operation In both animals active peristalsis was seen in the stomach and waves of contraction were seen to cross the pylorus Barium meal studies showed that the stomach emptied in 2½ to 3 hours

The appetite and capacity for food of the dogs gradually increased after operation After 12 weeks they were able to manage a normal sized meal

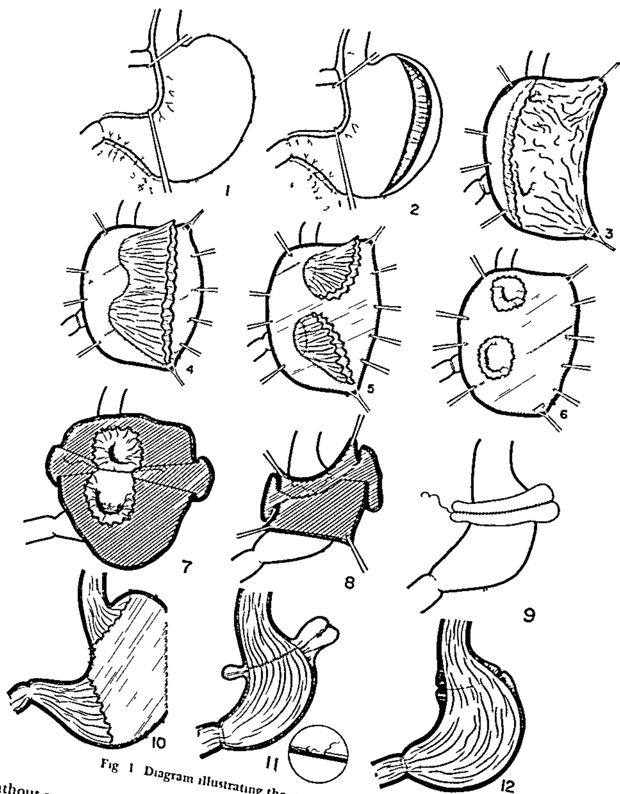


Fig 1 Diagram illustrating the steps in the operation

without any trouble or apparent discomfort. It seemed likely that the stomach gradually increased in size after operation

Repeated tests on the gastric juice showed that no acid was produced in response to food or histamine injection A series of 10 dogs at least 6 weeks after their operation was given 40 mg of histamine base, in beeswax daily for 4 weeks[9] They were then killed Both the stomach and duodenum in all

the animals were free from ulceration or inflammation Five control dogs, similarly treated, developed duodenal ulcers, 2 of which perforated

Of the 10 dogs which were kept longer than 12 weeks after their operation, 4 gained from 0 5 to 1 kg. in weight, 5 regained and maintained their pre operative weight and 1 lost 0 5 kg in weight

SUMMARY AND CONCLUSION

The operation of gastric mucosal excision produces an achlorhydria and protects against histamine induced ulceration.

The antrum and pylorus are preserved with their nerve supply intact and after operation there is a gradual dilatation of the stomach There is therefore little interference with the storage function of the stomach

The operation is not difficult to carry out and has a wide margin of safety Only a clinical trial of this operation in the treatment of peptic ulcer will show whether the incidence of postgastrectomy troubles is lessened On theoretical grounds one would expect this to be so

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EXPERIMENTAL PRODUCTION OF PEPTIC ULCERATION *

A New Method

O H Silberman, H T G Williams, W Pisesky
and R C Harrison

Many operative procedures are presently being advocated and used for the surgical treatment of peptic ulcer and there is no unanimity as to which are most satisfactory This subject has been investigated in the laboratory on many occasions but it has been difficult to assess surgical procedures when tested against the experimentally induced peptic ulcer The standard surgical procedures have been tested against gastric pouch secretion [1] or the development of peptic ulcer The former is a measure of blood borne gastric stimulants and so tells only part of the story

The experimental production of ulcer has introduced factors which tend to distort the comparative value of the ulcer protecting surgical procedure For example histamine [2] (which may not have any bearing on clinical peptic ulcer) acts by stimulating the parietal cells directly and so if this method of ulcerogenesis is used all procedures employing resection of part of the parietal cell mass will show a disproportionately low incidence of ulcer [3, 4] The Exalto-Mann Williamson procedure interferes seriously with the animal s nutrition (which favors ulcer development) and may also result in increased parietal cell secretion [5] Here again a bias will result in favor of procedures which interfere minimally with nutrition or which involve parietal cell excision

Ideally the ulcerogenic preparation should produce ulcers regularly exogenous drugs should not be employed to stimulate one specific phase of secretion the animal s nutrition should be maintained and there should be no anatomical or physiological alteration between the vagi corpus antrum or pylorus We believe this preparation offers some advantages over those previously employed

METHOD

Only male dogs were used At operation the duodenum was transected between the pylorus and the entrance of the biliary and pancreatic ducts A 15 to 40 cm length of terminal ileum was isolated and after continuity of the terminal ileum had been reestablished the ileal segment was interposed isoperistalticly between the first and second portions of the duodenum The bile and pancreatic ducts were not transected but of course were moved distally along with the second portion of the duodenum (Fig 1) All anastamoses were two layer 3 0 chromic catgut

Starting on the second postoperative week the stools were tested once weekly for blood If the animals did not die of hemorrhage or perforation they were sacrificed when their stools consistently showed occult blood In our series this interval between operation and postmortem varied from 5 to 24 weeks

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* From the McEachern Laboratory and the Department of Surgery University of Alberta Edmonton Aided by grants from the National Research Council of Canada and the City of Edmonton Civic Employees

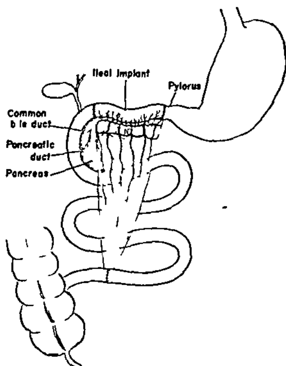


Fig 1 The ileal insert

When we were satisfied that this was truly an ulcerogenic procedure we investigated the possibility that the ileal insert might stimulate parietal cell secretion, or interfere with the normal inhibitory action of the duodenum on parietal cell secretion [6]

Heidenhain pouches were constructed in 4 animals After 4 to 6 weeks the 24 hour pouch secretions were collected for 2 weeks and acid and chloride output determined These were again determined 4 to 6 weeks following transplant of an ileal loop which was 35 cm in length

RESULTS

Of 9 dogs which had the ileal transplant alone 7 developed large deep ulcers, and 1 of these died of perforation One died of a massive gastrointestinal hemorrhage, yet an ulcer was not demonstrated The remaining dog had occult blood in his stools for 2 weeks prior to sacrifice, but only an erosion was found at postmortem Most ulcers were situated in the ileal loop close to the first portion of the duodenum and some involved both the duodenum and the loop Two dogs had 2 ulcers in the ileal loop It is of course difficult to know just when these ulcers developed, but from stool studies we believe they did so from the 4th to the 17th week Thus a considerable period of observation is necessary

With respect to the effect of this procedure on Heidenhain pouch secretion, our results were equivocal Two animals showed a decrease in pouch secretion, and 2 an increase

DISCUSSION

We were unable to demonstrate any correlation between the length of the implant and the tendency to ulcer development in it

In considering the pathogenesis of these experimental ulcers the following possibilities must be considered

1 The preparation increases acid secretion by eliminating or reducing the normal inhibitory effect of the duodenum or by stimulating acid secretion in some manner In 2 rather similar procedures described by Wangensteen[6] an increase in pouch secretion resulted but no ulcers were described He used a jejunal rather than an ileal loop As noted we failed to observe any consistent effect on pouch secretion following ileal transplant We do not believe this to be a significant factor

2 The reflux of bile pancreatic secretion and succus entericus does not reach the proximal anastomosis of the implant to successfully buffer dilute and neutralize gastric acid This is probably due to the length of the interposed segment of ileum and reabsorption of the alkaline secretions by the implant

3 The ileum lacks resistance to acid peptic digestion It has been reported that as the stomach is anastomosed to progressively lower segments of the intestinal tract there is an increasing incidence of stomal ulcers which has been attributed to a gradient of susceptibility to ulceration It has been shown however that in the absence of neutralizing secretions ulcers form with equal facility in the duodenum jejunem and ileum of the dog which lacks Brunner s glands[8]

The reason for occurrence of ulceration in our preparation is probably due to the loss of the alkaline pancreatic and biliary secretions in the postpyloric area

CONCLUSIONS

A new experimental method has been described which results in the formation of peptic ulcer without interfering with the stomach or pylorus the introduction of an acid stimulating drug without external loss of gastrointestinal secretions and without interfering with the animal s nutrition

This preparation is being used to test the many presently advocated surgical procedures for ulcer We feel that it will be valid for this purpose

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INTRAINTESTINAL AND GASTRIC pH CHANGES FOLLOWING BILLROTH GASTRECTOMIES *

PETER A SALMON, HARLAN D ROOT, CONRAD B JENSON, NORMAN W CRISP, KAMIL IMAMOGLU AND OWEN H WANGENSTEEN

Goligher [1] and Ordahl [2] have reported the late recurrence rates following the Billroth I operation for duodenal ulcer as 17 to 28% and 2 to 6% following the Billroth II gastric resection Assuming little disparity in the sizes of resection the essential difference between them lies in the manner of alimentary reconstitution It has been suggested that following resection of the pylorus and antrum with gastroduodenal reconstruction the stoma and duodenum proximal to the ampulla of Vater are bathed directly by the unbuffered secretions of the gastric remnant [1] in contrast to the situation following the Billroth II procedure where the stoma and efferent limbs are perfused by the highly alkaline content of the duodenum

Postoperative studies at the University of Minnesota Hospitals [3] have shown that a significant number of patients with Billroth I and Billroth II resections secrete gastric juice with pepsin concentrations in the duodenal ulcer range However, whereas none of the patients with Billroth II gastrectomy have intragastric free acid (pH of 3 5 or below), 10% of those with the Billroth I procedure secrete free acid and 20% have pH values of 3 5 units or below in unstimulated night gastric secretion The increased proteolytic activity of pepsin at pH values below 3 5 units is well established Therefore it appears *probable that the higher recurrence rate of peptic ulcer following the* Billroth I as compared with the Billroth II operation is largely referable to the presence of unbuffered acid gastric juice at the gastroduodenal stoma and in the first segment of the duodenum

METHOD

Under intravenous nembutal anesthesia adult mongrel dogs of both sexes weighing 20 to 45 lb, had 50% Billroth I or Billroth II gastrectomy performed In the first group reconstruction was by Hofmeister closure of the lesser curvature with end to end gastroduodenostomy In the Billroth II group a short loop, antecolic, isoperistaltic, gastrojejunostomy was performed after Hofmeister closure of the lesser curvature The stomas were of similar size approximately 2 5 to 3 cm in diameter

Rubber mushroom catheters were placed in the stomach 4 to 5 cm above the anastomosis and in the duodenum or efferent jejunal limb 1 cm distal to the stoma The catheters were wrapped in the omentum and brought through separate plastic cannulas in the lateral abdominal walls They were occluded except during removal of specimens

All dogs were kept on the regular kennel routine until the start of the experiment on the tenth postoperative day Five hundred milliliters of normal saline were given to each animal daily

In preparation for the experiment, the dogs were fasted 18 to 21 hours except for water An initial fasting specimen was then obtained from each

* From the Department of Surgery University of Minnesota Medical School Minneapolis
Supported by USPHS Grants RG 4780 and RG 1028

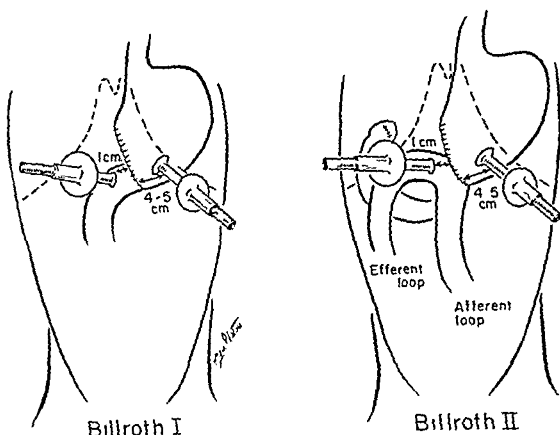


Fig 1

catheter and then 0 5 to 1 mg of aqueous histamine base was injected subcutaneously Specimens were obtained every 15 minutes for 2 hours One to 2 ml specimens from each catheter were collected in separate bulb syringes and pH values were immediately determined with pH test paper (p Hydrion) † Four or 5 complete experiments were carried out on each animal but only a single experiment on any given day

A total of 22 animals was studied 10 were Billroth II and 12 were Billroth I preparations

RESULTS

A total of 556 pH determinations was done, 260 were obtained from Billroth I and 296 from animals with Billroth II operations Enteric and gastric pH values each constitute ½ of the total number of determinations from each group

Figure 2 compares duodenal to jejunal pH determinations through the ranges of 0 to 3 0 to 3 5 and 0 to 4 0 units In general, pH values of 4 0 or below occurred much less frequently in the Billroth II than the Billroth I group Only 2% of specimens from dogs with the Billroth II procedure fell within the range of 0 to 3 while 10% of those from animals with the Billroth I operation were within this range Similarly 4 6 and 16 8% of the values from the Billroth II group fell in the pH ranges 0 to 3 5 and 0 to 4 0 respectively while 18 2 and 35 4% of determinations from the Billroth I group fell in these ranges Eighty three per cent of the Billroth II samples were over pH 4 0 whereas only 64% of Billroth I values were similarly elevated

† Micro Essential Laboratory Brooklyn

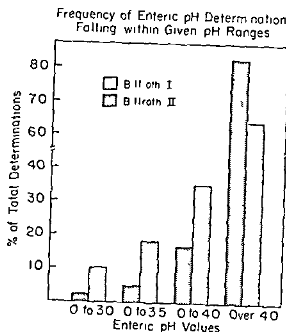


Fig 2

Table 1 Mean and Range Values for Intragastric pH within Given Enteric pH Ranges

	INTESTINAL	pH RANGE	0 TO 3 0	0 TO 3.5	0 TO 4 0
Billroth I	Gastric pH	Mean	1 7	1 9	2.5
		Range	1 4–1 8	1 4–3 0	1 4–3 7
Billroth II	Gastric pH	Mean	1 7	2 0	2 1
		Range	1 0–2 0	1 0–3 0	1 0–4 0

The data in Table 1 compares gastric pH values found in the various enteric pH ranges and shows that there is little difference between them

DISCUSSION

While the use of a 50% Billroth I or Billroth II gastrectomy without a complementary procedure is uncommon in the clinical treatment of peptic ulcer, its use in this experiment is justified by the necessity of retaining a large enough gastric remnant to secrete a potent acid gastric juice in the absence of the peptic ulcer diathesis

Postanastomotic enteric pH values following Billroth I gastrectomy fall more frequently within the range of maximum peptic activity than after Billroth II resection The difference in the incidence of low enteric pH values observed experimentally between the two types of gastrectomy is striking, particularly since the size of resection, stoma size, and stimulation have been controlled It is known that potent acid buffering mechanisms exist in the duodenum which seem to be better utilized by the Billroth II reconstruction The obligatory passage of bile, pancreatic juice, and succus entericus through the stoma after the Billroth II operation may explain this difference The effectiveness of this buffering mechanism is doubly significant in light of the probable decreased susceptibility to peptic ulceration of the duodenal mucosa as compared to that of the jejunum

Other evidence from our data supports the concept of more effective acid neutralization after Billroth II than Billroth I resection While gastric pH

values were almost identical in the three ranges of pH studied, regardless of type of resection the small number of determinations from the Billroth II group in which enteric pH values were below 4 0 and especially below pH 3 5 indicates more rapid neutralization of acid at the stoma and in the efferent jejunal loop The Billroth I reconstruction seems less effective in utilizing the available buffering juices because the pancreatic juice and bile enter the duodenum distal to the stoma and therefore regurgitation of alkaline juice must be in an antiperistaltic manner

SUMMARY

1 Following aqueous histamine stimulation 556 gastric and intestinal pH determinations have been carried out on dogs with 50% Billroth I and Billroth II gastric resections

2 Lowering of gastric pH was comparable in the two groups.

3 A much greater percentage of determinations from animals with Billroth I operations had enteric pH values below 4 0 units than were obtained from the Billroth II group

4 It is suggested that the Billroth I type of reconstruction gives less adequate protection from recurrent peptic ulceration in that the available buffering mechanisms are spatially removed from the stoma and first portion of the duodenum thereby allowing unbuffered acid peptic juice to bathe the area

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ABSOLUTE IRON ABSORPTION FOLLOWING SUBTOTAL GASTRECTOMY IN THE DOG *

NOBUYUKI SHIOYA SURYAKANT TALSANIA RUSSELL C BRIGGS AND SAMUEL R POWERS JR.

Post gastrectomy anemia has been one of the causes of morbidity after partial gastrectomy Several clinical studies have been reported on this problem in particular and also in connection with the malabsorption syndrome which may follow gastrectomy Experimental studies have also been reported recently on iron absorption after gastrectomy using isotopic iron

In this study we have attempted to determine the absolute iron absorption in dogs after partial gastrectomy using Fe^{59}

* From the Department of Experimental Surgery Albany Medical College of Union University

METHOD

Eighteen healthy mongrel dogs were chosen for the study and were divided into 3 groups Group 1 Five normal animals without any operation Group 2 Partial gastrectomy with Billroth I anastomosis plus splenectomy was performed on 6 dogs one of which underwent a Billroth I without splenectomy Group 3 Partial gastrectomy with Billroth II anastomosis plus splenectomy was performed on 7 dogs 1 of which underwent a Billroth II without splenectomy

The extent of the resection was estimated to have been between 67% and 75%

Three months after the procedure, each dog was fed 10 μc of radioactive Fe^{59} (1 μc/0 5 mg Fe^{59} as ferric chloride) by nasogastric tube

Hemoglobin determination at the time of the feeding revealed an average of 9 0 gm Control group animals were made anemic by blood loss during the 3 months period Reticulocyte count was determined before the feeding and 3 days after the feeding The dogs were maintained on iron free diet before and during the experiment After the feedings the animals were placed in individual metabolism cages and the total excreta was collected over a 7 day period The total 7 day specimen was then made homogenous by means of a Waring blender Aliquots of 3 cc from each of the 7 day specimens were then counted for radioactivity by the deep well scintillation technique

RESULTS

The results shown in Table 1 are expressed in percent of the radioactive iron removed from the amount given through the nasogastric feeding The average percentage of Fe^{59} retained by 5 normal animals was 49 7% Group 2 (6 animals with Billroth I gastrectomy) retained 59 8% as an average, however, 2 animals among those 6 showed a marked increase in absorption Seven animals with Billroth II anastomosis Group 3, showed an absorption from 13 1% to 85 5% as shown in the table The average was 42 8%

DISCUSSION

In the early stage of this study the percentage absorption of most animals was found to be close to 50% without any significant difference between the 3 groups Occasional divergence from this figure was considered probably due to our lack of familiarity with the technique, however, after the completion of a series of eighteen dogs the following facts were noticed 1) all animals in the control group (Group 1) showed nearly 50% absorption, 2) Group 2 showed a percentage absorption not significantly different from those of Group 1, 3) Group 3 showed a complete lack of uniformity though the average figure revealed a questionably significant decreased absorption

In considering the significance of these results, the possibility of a technical error is unlikely because of the uniformity of the values in the control group

Animals after Billroth I operation seem to have a normal iron absorption

Various percentage absorption among animals with Billroth II operation may be caused by the individually altered function of the gastrointestinal tract after this type of surgery Whether this is a part of the vicious cycle of postgastrectomy malabsorption in general or the result of the effect of this

Table 1

NO	HGB	% ABSORPTION	TYPE OF OPERATION
GROUP 1			
1	8.5	57.2	None
2	8.0	50.1	None
3	8.5	52.0	None
4	9.0	43.5	None
5	11.5	45.7	None
	Average	49.7	
GROUP 2			
6	8.5	82.5	B I with splenectomy
7	7.5	54.5	B I with splenectomy
8	8.0	44.7	B I with splenectomy
9	7.5	52.0	B I with splenectomy
10	11.0	92.2	B I with splenectomy
11	13.0	33.5	B I only
	Average	59.8	
GROUP 3			
12	9.0	85.5	B II with splenectomy
13	9.0	56.1	B II with splenectomy
14	9.0	19.6	B II with splenectomy
15	8.0	48.1	B II with splenectomy
16	9.5	63.8	B II with splenectomy
17	—	14.1	B II with splenectomy
18	—	13.1	B II only
	Average	42.8	

type of operation purely on an iron absorption mechanism, is to be investigated further

This study does not show the utilization of the iron after absorption

The marked variation of percentage of absorption in Group 3 and to some extent in Group 2 suggests that some factors besides gastrectomy, such as gastric emptying or iron reserve, are more important in determining the total iron absorption

SUMMARY

Absorption of Fe^{59} was studied in 18 dogs Normal dogs showed an average of 19.9% absorption Those which had Billroth I operation showed an average of 59.8% absorption Those which had Billroth II operation showed an average of 42.8% absorption with a marked divergence of the values

This study showed no statistical difference of average absorption between control group Billroth I group, and Billroth II group

THE ROLE OF STOMAL SIZE IN THE POSTGASTRECTOMY DUMPING SYNDROME *

WILLIAM SILEN, BEN EISEMAN AND WILLIAM H BROWN, JR

The rapid introduction of hypertonic solutions into the duodenum or jejunum produces a decrease in plasma volume and symptoms characteristic of the postgastrectomy dumping syndrome [1 2 3] Since the normal stomach with an intact pylorus releases hypertonic solutions into the small intestine very slowly attempts have been made to reproduce normal slow gastric emptying by constructing a small gastro enteric stoma with the hope that undesirable postgastrectomy symptoms might be avoided [4]

The role of stomal size in the pathogenesis of the dumping syndrome has been evaluated in this study by measurement of plasma and blood volume changes in partially gastrectomized dogs with gastrojejunal anastomoses of varying size

METHOD

Seven dogs underwent a two thirds Billroth II gastrectomy In 4 animals (Group 1) a wide stoma (8 to 10 cm in diameter) utilizing the breadth of the transected stomach, was created at the first operation Conversely, a small stoma equal to the diameter of the jejunum and varying from 1 5 to 2 cm was created initially in the remaining 3 animals (Group 2) Following a suitable recovery period (1 to 3 weeks), two studies of plasma and blood volume responses to oral hypertonic glucose solution were carried out, 7 days apart Reoperation of all animals was then performed and the size of the stoma reversed without resection of additional stomach or jejunum (Fig 1) After a suitable rest period, the response of the blood and plasma volume to the glucose test meal was restudied twice with the altered stomal size Each animal thus served as his own control

All studies were carried out following an overnight fast Blood and plasma volume determinations were made before, and at 30 and 60 minutes after the administration of 100 ml of 50% glucose in water by means of a Levine tube

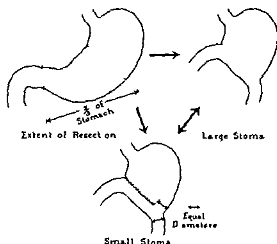


Fig 1 Operative procedures performed on dogs in Groups 1 and 2

* From the Department of Surgery University of Colorado School of Medicine and the Denver Veterans Administration Hospital With the technical assistance of Mr Fred O Stoll

placed in the midesophagus The animals were maintained in the standing position throughout the test Each of the 3 plasma and blood volume determinations were carried out in identical manner as follows A preliminary sample of blood was drawn for background count One milliliter of a 1 500 standard solution of human serum albumin I131 (RISA) (Abbott Laboratories) was injected into the femoral vein using a B D Cornwall constant delivery syringe Ten minutes were allowed for thorough mixing of the RISA within the blood stream and a second sample was drawn Utilizing this technique with a minimum of 20 000 counts per sample on an R C scientific well type scintillation counter Hlad and Tanz [5] in our laboratory have found an error of only ±2%

RESULTS

The pertinent mean changes in both blood and plasma volume for both groups of dogs are shown in Table 1

In all but 3 instances in which there were slight rises the plasma volume decreased appreciably 30 minutes following instillation of the hypertonic glucose solutions as indicated in Figure 2 Blood volumes similarly decreased

Group 1 (large stoma to small) although there was a wide individual animal variation in the plasma volume changes following the test meal the pattern remained constant with a mean plasma volume diminution of 9% with a large stoma and 10 4% with the small The whole blood volume changes paralleled this response

Group 2 (small stoma to large) in this group in which the order of stoma size was reversed the identical response pattern was noted The mean plasma volume decrease with the small stoma was 9 8% compared to a decrease of 4 3% with the large stoma As in Group 1, the blood volume changes paralleled this alteration

Inasmuch as reversing the order of stomal size did not alter the volume response the significant fact remains that the mean plasma volume decrease

Table 1 Mean Decrease in Plasma and Blood Volume Following Oral Administration of Hypertonic Glucose Solution

	GROUP 1		GROUP 2 (SMALL TO LARGE)		MEAN	
	PLASMA	BLOOD	PLASMA	BLOOD	PLASMA	BLOOD
Small Stoma	10 4	11 9	9.8	7.2	10.3	9.5
Large Stoma	9 0	7.8	4.3	2 6	6 7	6.8

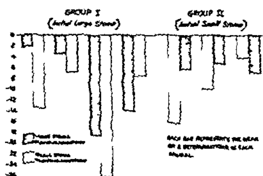


Fig 2 Percentage decrease in plasma volume following the administration of hypertonic glucose solution to partially gastrectomized dogs

in all of the animals studied with small stoma was 10 3%, while there was but a 6 7% over all mean diminution when large stomas had been created

DISCUSSION

Contrary to expectations, it is evident that under these experimental conditions stomal size does not appreciably alter the plasma or blood volume response to the hypertonic glucose solution test meal In fact, in most of the experiments there was a greater decrease in plasma volume when a small stoma gastrojejunostomy was present Controversy as to the role that stomal size may play in gastric emptying has existed for many years, for it was to preserve the reservoir function of the gastric remnant that the Hofmeister valve was originally devised[6] Later it was maintained that the diameter of the jejunum, not the stomal size, was the limiting factor More recently, Zollinger[4] has again advocated creation of a small stoma to delay gastric emptying and to avoid postgastrectomy symptoms

Although the results of these experiments cast doubt upon the importance of stomal size in altering the [illegible]
that the consistency of the te [illegible]
An aqueous solution such as [illegible]
through a small stoma, whereas food in particulate form might be impeded Despite these considerations, it is significant that diminution of stomal size did not minimize the plasma volume response to hypertonic glucose solution

SUMMARY

1 The role of stomal size in the production of the postgastrectomy syndrome has been evaluated in partially gastrectomized dogs with gastrojejunal stomata of varying size by measuring alterations in blood and plasma volume following the oral administration of hypertonic glucose solution

2 The experimental data indicate that stomal size is unimportant in affecting the magnitude of plasma and blood volume change under these experimental conditions

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A METHOD OF DUODENAL STUMP CLOSURE WITH INTACT OMENTUM *

ROBERT A. HARDIN, CHIEN SHENG SU, AND J. S. BATTERSBY

Closure of the duodenum for either a perforated ulcer or at the time of a gastric resection for chronic duodenal ulcer can be difficult and lead to a fatal outcome unless a satisfactory and safe closure is obtained. This study was undertaken to determine whether the omentum alone could be used to effectively close the duodenum.

METHOD

Mongrel dogs weighing between 12 and 18 kg were placed on *nil per os* for 8 hours prior to surgery. The dogs were anesthetized with intravenous sodium thiopental and subsequent anesthesia of ether administered by a cuffed endotracheal tube. Respiration was controlled and oxygen administered at the rate of 3 L/min. Aseptic surgical technique was used. The abdomen was opened in the midline to expose the stomach and duodenum. The duodenum was transected 1 cm distal to the pylorus and a 40% gastrectomy completed. The gastrointestinal continuity was reestablished with a Hofmeister type of gastroenterostomy.

The duodenal stump, however, was managed as follows. In Group 1 (Fig. 1 B) the duodenal stump was closed by inserting a quantity of omentum that was completely severed from its blood supply. The omentum, which was sufficient to mechanically occlude the duodenum, was carefully sutured in place. In Group 2 (Fig. 1 A) the omentum was sutured into the open end of the duodenum; however, a ligature was placed about the omentum to occlude its blood supply. The omentum which was sufficient to completely

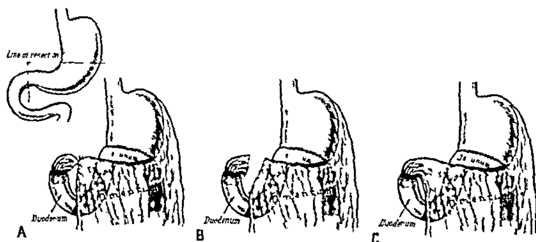


Fig. 1. A. Omental blood supply ligated. B. Free omental graft. C. *Intact omentum.*

* From the Department of Surgery, Indiana University School of Medicine, Indianapolis.
Aided by grant from the Riley Memorial Association.

fill the opening in the duodenum was sutured in place In Group 3 (Fig 1 C) the omentum was placed in the open duodenal end in sufficient amount to mechanically fill the duodenal opening The omentum was carefully sutured to the circumference of the cut edge of the duodenum but care was exercised to preserve the blood of the omentum The procedure for Groups 2 and 3 was identical except for the control of the blood supply to the omentum The postoperative management of each group of dogs was identical Each animal was placed on a liquid diet for 4 days after which a diet of kennel rations was resumed

RESULTS

In Group 1 animals (Table 1), 5 dogs were treated as outlined In 4 of the animals, death occurred in 24 to 36 hours In each instance, death was due to peritonitis from a leaking duodenal stump The omentum showed necrosis and liquefaction so that it no longer acted as an effective closure for the duodenum One animal lived This dog was sacrificed at 9 days The autopsy disclosed that the duodenal stump had become adherent to the abdominal wall and undersurface of the liver for an effective closure The results of this method of duodenal closure indicated failure in 4 out 5 animals

In Group 2 (Table 2), 5 animals were used The omentum was placed into the duodenum and carefully sutured around the circumference of this bowel The omentum was then ligated proximal to the duodenum but allowed to remain attached to the stomach In this group of animals, 4 died within 36 hours In each, the autopsy indicated leakage from the duodenal stump with peritonitis The omentum was necrotic and liquefied One animal survived An autopsy after 8 days indicated that adjacent small bowel had become adherent to the duodenum and effectively closed the open bowel

In Group 3 (Table 3), the basic operative procedure was identical to Groups 1 and 2 except that the omentum was sutured into the open duodenum and care exercised to maintain the normal blood supply to the omentum In this group 10 dogs were used Eight of the animals survived but were sacrificed to study the healing process at intervals of from 4 days to 9 weeks

Table 1 Free Omental Graft (Fig 1 B)

NO	DATE OF OPERATION	DATE OF DEATH OR SACRIFICE	POSTMORTEM FINDINGS
1	7 18 58	7 19 58	Peritonitis due to leakage from stump
2	7 19 58	7 21 58	Peritonitis due to leakage from stump
3	7 21 58	8 10 58†	Duodenal stump adherent to anterior abdominal wall and liver No gross presence of omentum visible
4	7 25 58	7 27 58	Peritonitis due to leakage from stump
5	8 1 58	8 3 58	Peritonitis due to leakage from stump

† Sacrificed

Table 2 Occluded Blood Supply of Omentum (Fig. 1 A)

NO	DATE OF OPERATION	DATE OF DEATH OR SACRIFICE	POSTMORTEM FINDINGS
1	11 12 57	11 13 57	Peritonitis due to leakage from stump
2	2 20 58	3 19 58†	Viable omentum and loops of small bowel had sealed off lumen
3	2 28 58	3 1 58	Peritonitis due to leakage from stump
4	3 11 58	3 13 58	Peritonitis due to leakage from stump
5	3 13 58	3 14 58	Peritonitis due to leakage from stump

† Sacrificed

Table 3 Intact Omentum (Fig 1-C)

NO	DATE OF OPERATION	DATE OF DEATH OR SACRIFICE	POSTMORTEM FINDINGS
1	11 1 57	11 8 57	Omentum intact No leakage
2	11 5-57	11 19 57	Omentum intact No leakage
3	12 4 57	12 27 57	Omentum intact No leakage
4	12 11 57	12 12 57†	Peritonitis due to leakage from stump
5	12 18 57	12 19 57†	Peritonitis due to leakage from stump
6	1 9 58	2 10 58	Omentum intact No leakage
7	1 16 58	2 24 58	Omentum intact No leakage
8	1 16 58	3-4 58	Omentum intact No leakage
9	3 17 58	3 31 58	Omentum intact No leakage
10	3 17 58	3 31 58	Omentum intact No leakage

† Death

In each case there was no evidence of leak, the omentum was healthy and the duodenal stump showed healing (Fig 2) A study of the microscopic sections indicated that healing of the duodenum had been completed by the fourth week with mucosa completely covering the duodenal stump There were 2 deaths in this group The autopsy of each indicated two



Fig 2 Microsection demonstrating healing of duodenal stump A Omentum B Duodenal mucosa

important points In 1 animal the blood supply of the omentum was inadvertently occluded by overzealous suturing of the omentum to the duodenum In the second animal there had been insufficient omentum used so that it could not act as an effective mechanical barrier to occlude the duodenal opening

When the deaths of these experimental animals are studied for each of the 3 groups it is apparent that omentum which has either been severed from its blood supply or had its blood supply ligated functions in an identical manner There is liquefaction and necrosis of the omentum with leakage of the duodenum and subsequent death of the animal A second factor as demonstrated in the Group 3, animal 4, suggested that the duodenal opening had to be closed by a sufficient amount of omentum to mechanically occlude the opening and prevent leakage of bile and pancreatic juice

When the survival animals in each group are studied there again appears two important factors In Groups 1 and 2 the survival of 1 animal each was the result of a *chance* adherence of bowel or adjacent viscera to seal the duodenum This occurred in about 20% or an unacceptable survival rate In Group 3 animals it was apparent that when there was living omentum in sufficient quantity all animals survived

In the experimental animal, these 3 groups of dogs indicate that the omentum can be used to effectively close the duodenal stump if the omentum is used in sufficient quantity to efficiently block the duodenal opening and if the blood supply to the omentum is not disturbed A free omental graft or omentum which has had its blood supply occluded can not be used to successfully close a duodenal stump

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STUDIES IN POSTOPERATIVE ILEUS INTESTINAL MOTILITY AS REFLECTED IN THE PROPULSION OF RADIOPAQUE MATERIALS *

JASPER L MCPHAIL, JAMES D HARDY, J HAROLD CONN, JOSEPH SCHOR, AND SAM ROBINSON

It is the common impression that intestinal motility, especially as regards forward propulsive activity, is largely absent immediately following major abdominal surgery The purpose of this study was to examine the validity of this impression

METHOD

The study methods selected were as follows 1) for cases in which the stomach was not entered, 30 cc of barium sulfate solution was instilled into the stomach per Levin tube at the end of surgery 2) when the stomach was entered, a small radiopaque marker was left in the lumen at the time of closure A flat plate of the abdomen was taken 4 and 24 hours postoperatively to determine the progress of the radiopaque material The radiologists reported the position of the radiopaque material as being in the stomach, jejunum, ileum cecum, ascending, transverse or descending colon, sigmoid or rectum An average of 50 mg of meperidine hydrochloride was given every 4 hours as necessary for postoperative analgesia All patients were ambulated within 24 hours Bowel sounds were recorded as present or absent

RESULTS

Fifty patients were studied The data were summarized as to each patient's age, sex, operation performed, radiopaque material used, progression along gastrointestinal tract at 4 and 24 hours, and the presence or absence of clinical bowel sounds Of the 50 patients, 44 were males, and 6 were females The ages varied from 25 to 71 years, with an average age of 51 years The types of operations used in this study were as follows subtotal gastrectomy with gastrojejunostomy, 24 cholecystectomy, 10, herniorrhaphy, 5, gastrojejunostomy with vagotomy 5 partial colon resection, 3, appendectomy, 1, gastrostomy with polypectomy, 1 and cholecystojejunostomy, 1

In the 19 patients in whom barium was used, in 4 hours it had progressed to the jejunum in 8 instances to the ileum in 4 and had remained in the stomach in 7 In 24 hours the media was in the terminal ileum in 4, in the cecum in 5 in the ascending colon in 1, transverse colon in 7 and sigmoid in 2 Bowel sounds were still absent in 4 patients at 24 hours, present in 15 patients at 24 hours and present in only 4 patients at 4 hours

In the 31 cases in whom the radiopaque markers were placed at the time of surgery, the marker had progressed to the jejunum in 22 patients in 4 hours, to the terminal ileum in 7, to the cecum and still in the stomach in only one In 24 hours the markers were in the sigmoid in 5 cases, transverse colon in 3 ascending colon in 1, cecum in 9, terminal ileum in 11, jejunum in one and remained in the stomach in only one Seventeen of these patients

* From the Departments of Surgery Veterans Administration Center and University of Mississippi Medical Center Jackson Supported by National Institutes of Health Grant Number RG 4745(C)

had absent bowel sounds at 24 hours The other 14 had hypoactive bowel sounds at 24 hours

COMMENT

This study indicated that propulsive intestinal activity is present long before bowel sounds can be heard clinically The common practices of gastric suction and the withholding of oral intake for 2 or 3 days following gastrointestinal operations may be necessary only occasionally, and in some subjects it may actually contribute to the patient s discomfort and aggravate electrolyte and nutritional difficulties

Ether anesthesia may result in nausea and vomiting in the early postoperative period The utilization of newer anesthetic agents combined with muscle relaxants and the administration of gaseous anesthetics through the endotracheal tube may further diminish the incidence of these complications

In a recent report Corbit[1] has stated that early ingestion of solid food following abdominal surgery may be a major factor in preventing abdominal distention and gas pains Craig, Wohl and Shuman[2] also have reported that the early institution of oral feedings can significantly reduce the amount of postoperative nausea and ileus

CONCLUSIONS

It is apparent from the present study that propulsive intestinal motility is usually present almost immediately following major abdominal operations This early activity cannot be correlated with bowel sounds In view of recent surgical advances including better preoperative preparation improved anesthesia, and early ambulation, traditional postoperative regimens often require reevaluation Postoperative gastric siphonage, the withholding of oral intake and prolonged parenteral fluid therapy is not necessary for many patients undergoing abdominal operations, and possibly should be reserved for only the occasional patient who clearly requires it

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THE FLUORESCEIN STRING TEST IN GASTROINTESTINAL HEMORRHAGE *

MITCHELL KARLAN AND DONALD W TRAPHAGEN

The localization of bleeding in gastrointestinal hemorrhage is unknown in 15 to 20% of the cases examined [1, 2, 3, 4, 5] This is so, even with all clinical laboratory and radiographic tests available To improve this percentage, a modification of the string test has been developed using a string marked with a radiopaque tape and fluorescein dye This test has been tried on more than 40 patients without any untoward complications

METHOD

The following equipment is needed for the examination

1 String (a thin 1/32 in gauze umbilical tape †) A radiopaque marker is imprinted longitudinally into the string for 7 ft and also cross marked with the same material at 1 in intervals A small 2 gm shot is attached to the end of the string to facilitate swallowing and passage through the pylorus

2 Fluorescein 5%—processed with sodium bicarbonate †† This sterile solution is not toxic and can easily be given in the same amounts as normal saline

3 An ultraviolet light or small mineralight †††

The string is moistened with water and the patient usually swallows it easily It is more readily tolerated than an intubation tube A flat film of the upper abdomen is obtained 2 to 3 hours later to determine the string s location The end of the string should be passed at least 2 ft beyond the ligament of Treitz It is easy to detect the course and progression of the string by noting the radiopaque markers and their relationship to the pylorus When the patient obviously is having active gastrointestinal bleeding 10 cc of fluorescein is injected into an antecubital vein After 5 minutes the string is removed It is examined first for evidence of gross blood and then taken to a darkened room and examined against a dark background with an ultra violet light

The point of bleeding is determined by matching the place where blood or fluorescein occurs on the string with its comparable location on the x ray film Because of the typical curvature of the string in the region of the duodenal C loop accurate anatomical localization of the bleeding point is readily obtained If the appearance of the dye on the string is not clearly seen then the fluorescein can be made strikingly apparent by immersing the string in a trough of water and examining it under an ultraviolet light

RESULTS

The string test was first tried in the animal research laboratory A bleeding area was established in an anesthetized dog by leading a catheter from one femoral artery to a point in the gastrointestinal tract Strings were

† Supplied by Johnson and Johnson Company New Brunswick New Jersey

†† Supplied by the C F Kirk Company New York City

††† Supplied by Ultra Violet Products Inc. South Pasadena California

* From the Department of Surgery The Ohio State University College of Medicine and the Medical Center Columbus

passed attached to long Cantor tubes and various bleeding points were selected on different dogs Another investigator using the described technique localized the one bleeding area taking radiographs injecting fluorescein dye and withdrawing the string

With the satisfactory laboratory results the test was then started on hospital patients with gastrointestinal hemorrhage The examination has been especially helpful in localizing bleeding from such upper gastrointestinal lesions as esophageal varices hiatus hernia and duodenal ulcers Three representative cases are presented

Case A *J A* a 31 year old white female was discharged 4 weeks ago after an uneventful cesarean section She had noted melena and hematemesis for 48 hours prior to this admission and her hemoglobin in the emergency room was 6 gm % Physical examination disclosed a pulse of 140 B P 90/60 and hepatosplenomegaly Her prothrombin time was 30% The admitting diagnosis was bleeding varices with portal hypertension Her past history was essentially normal Four pints of blood were given over a 6 hour period and her vital signs failed to improve A string was swallowed and a portable radiograph obtained of the upper abdomen immediately thereafter Ten cubic centimeters of fluorescein was injected and the string was removed 2 minutes later Radiographs showed that the string had passed into the fundus of the stomach No blood or fluorescein was noted on that part of the string which was in the esophagus The patient was prepared for and taken to surgery A bleeding duodenal ulcer was noted and a partial gastrectomy and vagotomy was done without unusual incident

In this case the string test demonstrated immediately that the location for the bleeding was lower than the esophagus It also spared the patient any further delay or trial at esophageal tamponade A negative test was thus very helpful

Case B *A V* a 48 year old white female was admitted with anemia and weight loss She had had an upper gastrointestinal series and barium enema 2 months before and these were both reported as normal Physical examination here was essentially unremarkable Her hemoglobin on admission was 8 5 gm % and her stools were Guaiac positive A string was passed in the evening and permitted to stay down overnight It was caused to advance 3 in every hour A flat film in the morning showed the string had passed into the ileum Fluorescein was injected and the string withdrawn The string was coated with blood from the stomach cardia on down No fluorescein dye was noted A repeat upper gastrointestinal series was requested It showed findings compatible with a linitis plastica which was confirmed at laparotomy

In this case active bleeding did not occur at the moment of the injection of the dye The slow oozing during the night was enough to coat the string and suggest a repeat upper gastrointestinal series with attention directed to the stomach

Case C *J K* a 15 year old white male was admitted with a hemoglobin of 7 gm % and a history of passing dark bloody stools for several days He had hematemesis the morning of admission An upper gastrointestinal series disclosed a large hiatus hernia and an intrinsic mucosal irregularity in the second portion of the duodenum A string was swallowed and 3 hours later a flat film of the abdomen showed the string to be in the first portion of the

jejunum. Ten cubic centimeters of fluorescein was injected and 3 minutes later the string was removed. Blood and fluorescein were noted in the area of the second portion of the dudoenum. Exploratory laporatory disclosed a primary duodenal carcinoma.

The string test in this case localized the point of active bleeding in a situation where 2 lesions were present. Either could have caused the source of hemorrhage. Total therapy was directed at the duodenal lesion. Thus, the surgeon did not have to worry about the possible postoperative hemorrhage from the hiatus hernia site.

DISCUSSION

Managing gastrointestinal hemorrhage is difficult. When the source of bleeding is unknown this problem becomes worse. It taxes the ingenuity of the clinician and the morbidity and mortality increase. The string test has been used in more than 40 cases of gastrointestinal hemorrhage during the past year. At first, it was used to corroborate suspected bleeding areas determined by radiographic examination. Thereafter, it was used when the bleeding point was unknown. In our series, no untoward complications or rebleeding have been caused by the test.

The examination discloses one of three possibilities: 1) the string may contain no blood or fluorescein. This indicates that there is no active bleeding in the area passed by the string or that the string is above the point of hemorrhage (case A). 2. The string may contain only blood and no fluorescein. This indicates that active bleeding is not occurring at the moment of the test but that bleeding had occurred in the past in that area. It can also indicate regurgitated blood from a point distal to the area passed by the string (case B). 3. The string may contain blood and also demonstrate the fluorescein dye. This indicates active bleeding and the site of the dye shows the point of origin of the hemorrhage (case C).

The fluorescein string test is no panacea but rather an aid which the clinician can use when the standard laboratory and radiographic examinations have been of no help. It is recommended that this test be given further clinical application.

SUMMARY

The fluorescein string test is presented as an aid in localizing gastrointestinal hemorrhage.

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A SMOOTH MUSCLE STIMULATING SUBSTANCE RELEASED FROM THE INTESTINE FOLLOWING ACUTE ARTERIAL OCCLUSION *

DONALD M PERLMAN AND JACK W COLE

It has been shown that spasm of the intestine occurs following acute interruption of the arterial blood supply One of the earliest descriptions of the phenomenon was by Litten (1875),[1] who experimentally ligated the superior mesenteric artery in dogs He noted that the ischemic intestine became "rippled and contracted while assuming a bluish white color

Laufman and Method (1947)[2] acutely ligated the artery and vein supplying a segment of dog s intestine In addition to the change described above, *they found that release of the occlusion prior to complete relaxation of the* spasm produced a phase of "reactive hyperemia ' in which the contraction rings of the intestine gave way to a more organized and hyperactive peristalsis

In contrast with these effects, studies in which a segment of intestine is perfused with hypoxic blood show only a brief initial intestinal hypermotility followed by diminished intestinal tonus and motility[3]

Factors which might be implicated in the production of intestinal spasm from ischemia include 1) extrinsic autonomic nervous system reflexes 2) intrinsic neurogenic reflexes set up in the bowel itself, 3) activation or release of smooth muscle stimulating substances present in the blood or in the intestine, 4) production of abnormal substances, through the effect of ischemia, with possible smooth muscle stimulating ability, 5) direct effect of ischemia on muscle and nerve cells

Of special interest to us, was the possibility that release of a humoral physiologic substance might be responsible at least in part for the intestinal spasm One such substance now being widely investigated is 5 hydroxytryptamine (5 HT) *which is thought to participate in the mediation of intestinal* peristalsis[4]

In order to test the hypothesis that ischemia results in the release of a smooth muscle stimulating substance (possibly 5 HT) an experiment was set up in which samples of venous blood were obtained from an intestinal segment before and after occlusion of the arterial blood supply These samples were then bioassayed for smooth muscle stimulating effect

METHOD

Operative 12 large mongrel dogs were used twice each with a 2 week period of convalescence between operations Under anesthesia induced by intravenous sodium pentobarbital (25 mg /kg) the abdomen was entered through a midline incision After withdrawal of a control blood sample from a mesenteric vein, a 10 to 15 cm segment of small intestine was selected at random Following ligation of the marginal blood supply, the artery supplying the segment was isolated and an occluding ligature was passed around it Record was made of the change in tonus and motility of the ischemic segment and blood samples from the efferent vein were drawn at

* From the Department of Surgery Western Reserve University School of Medicine and the University Hospitals of Cleveland Supported in part by U S P H S Grant #RG 4268

varying intervals (10 seconds to 15 minutes) When the occluding ligature was released a venous flush sample was withdrawn as the arterial circulation was restored A final mesenteric vein sample was then obtained Preoperative venous blood samples were procured from a number of animals prior to anesthesia

On three occasions the serosal margins and neurovascular pedicles of the ischemic segment were cocainized in the attempt to abolish extrinsic *neurogenic stimuli*

Collection handling and bioassay of the blood samples were performed according to methods described by Gaddum Peart and Vogt (1949)[5] The blood was collected in siliconized equipment using heparin as an anticoagulant It was then centrifuged for 20 minutes at 1500 rpm or more to obtain the plasma which was decanted and stored at —17° C After thawing at room temperature 0 5 ml of plasma was diluted in a glass beaker with 5 ml of Jalon s solution to which atropine (1 mg /L) and chlorprophenpyredamine (1 mg /L) had been added Assay was carried out on uterine horns obtained from Sprague Dawley white virgin female rats weighing 200 gm to which stilbestrol (1 mg /kg) had previously been administered A smoked kymograph drum was used for recording

A standard dose of 5 HT 0 02 μg/ml was used to produce a standard contraction for comparison with contractions produced by plasma samples and as a measure of the stability and sensitivity of the uterine preparation

RESULTS

Motility the small intestine was generally quiescent *in situ* The changes in the ischemic segment were similar to those described by other investigators Blanching and cyanosis occurred consistently while well marked spasm was noted in 16 of the 24 experiments In 9 experiments there was considerable marginal hypermotility in the intestine adjacent to the ischemic segment Cocainization did not alter the onset or the degree of spasm which followed acute arterial occlusion

Bioassay Plasma samples were suitable for assay in 19 experiments These samples showed smooth muscle stimulating activity in almost all cases It is noteworthy that in 13 experiments the plasma obtained from the ischemic intestine produced a smooth muscle stimulating response greater than that produced by control plasma (Fig 1)

Description of Bioassay Response The response occurred after a latent period of less than 10 seconds rapidly reaching a peak in less than one minute and then subsiding gradually Following this the refractory period before the muscle regained full activity lasted from 3 to 10 minutes depending on the magnitude of the preceding contraction One feature of the response which occurred at times was a tendency for increased vigor of contraction with equivalent amounts of plasma sample while the 5 HT response was consistent In order to show that a false increase of activity in the ischemic sample was not produced by this reversal of control and ischemic samples was done several times (Fig 2)

Properties of the Smooth Muscle Stimulating Substance The stimulating response was not blocked by lysergic acid diethylamide a specific antagonist of 5 HT (Fig 3) At 0°C the plasma sample showed no activity but after warming for 5 to 10 minutes at 20°C most of the activity was present and

Kymographic Tracings

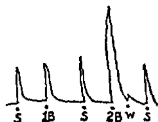


Fig 1 Comparison of control and ischemic plasma samples S represents application of the 5 HT standard (0 02 μg/ml) 1B is the control sample and 2B is the ischemic sample Note short latent period and *prompt rise to a peak*

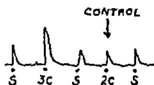


Fig 2 Demonstrates that ischemic sample 3C produces a greater effect than control sample 2C despite reversal of order of application



Fig 3 Effect of LSD on 5 HT standard and upon plasma samples LSD (0.2 μg/ml) on muscle 5 minutes 5 HT standard (S) is blocked Neither control sample 1 or ischemic sample 2 is blocked



Fig 4a Effect of room temperature on activity of plasma sample 4 First application shows long latent period then very small contraction Sample at 0° C Second application shows prompt powerful stimulation by sample after standing at room temperature for 20 minutes



Fig 4b Effect of room temperature on activity of plasma sample 1 Sample applied 10 minutes 22 minutes 34 minutes 62 minutes 2 hours and 44 minutes after thaw S is standard



Fig 5 Demonstrates destruction of activity of plasma sample 2 by trypsin Marks at point where 2 is applied represent wash in artefact

after 20 minutes it was at a maximum At the end of 2 to 3 hours a decay of activity was noted (Fig 4a, 4b) while after 24 hours at 20°C no activity was found Incubation with either trypsin or chymotrypsin abolished activity (Fig 5)

DISCUSSION

Our observation of changes in the intestine following acute mesenteric arterial occlusion agree in general with those previously reported [2-6] A relationship of extrinsic neurogenic reflexes with the intestinal spasm is not

Table 1 Comparison of the Properties of some of the Smooth Muscle Stimulating (Leiomyergic) Substances Found in Blood or Tissue Extracts

Substance	Effect on uterus	Effect on intestine	Inactivated by: Trypsin	Inactivated by: Chymotrypsin	Temperature	Remarks
Smooth muscle Stimulating factor (this experiment)	Contracts briskly	? Contracts	+	+	Incub 37° 2 hrs room temp 24 hrs	
Acetyl choline	Contracts	Contracts				Atropine inhibits
5 H T	Contracts and relaxes rapidly	Contracts				LSD specific inhibitor
Epinephrine / Nor epinephrine }	Inhibits (Physiol dose)	Inhibits (Physiol dose)				
Oxytocin	Contracts	Mild contraction	0	+		
Vasopressin	Contracts	Contracts	+	+		
Bradykinin	Contracts slowly	Contracts slowly	0	+		
Substance P	Moderately fast contraction	Contracts	+	+		
Histamine	Inhibits	?				Antihistamine blocks
Darmstoff	Contracts	Contracts	0	0		

ruled out by this experiment although spasm occurred despite cocainization of extrinsic nerves The preliminary results of bioassay indicate the presence of one or more smooth muscle stimulating factors in canine blood that are frequently increased under the conditions of these experiments Evaluation of active factor such as this is made difficult by the fact that when blood is removed from the body a number of substances which may interfere with bioassay are formed or released Results of the experiment rule out that the substance is identical with 5 HT, acetylcholine or histamine because of the use of specific blocking drugs against these agents The nature of the muscle response together with abolition of activity of the stimulating factor by chymotrypsin and trypsin distinguish this factor from certain other known smooth muscle stimulating substances such as oxytocin [7] bradykinin [8] or Darmstoff [9] Substance P,[10] and vasopressin [8] have properties similar to *the factor we studied but as yet we have no evidence of a definite relationship* A comparison of some of the more important known smooth muscle stimulating substances with the substance studied is shown in Table I It appears that *the factor is normally present in plasma and is increased in amount* under the condition of ischemia

SUMMARY

Twenty four experiments have been carried out in which a segment of canine small intestine was made ischemic by occlusion of its segmental artery In 16 experiments marked spasm of the ischemic segment was noted This spasm was not suppressed by cocainization of the extrinsic nerve supply Bioassay showed smooth muscle stimulating activity to be present in canine plasma under the conditions of our experiment In 13 out of 19 assays this activity was increased in plasma samples obtained from venous blood draining the ischemic segment The possibility that this smooth muscle stimulating factor plays a role in the pathophysiology of acute mesenteric arterial occlusion is suggested by these experiments and further studies will be undertaken to study a possible relationship

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THE RELATIVE EFFECTS OF ARTERIAL OCCLUSION AND VENOUS OCCLUSION ON INTESTINAL BLOOD FLOW *

M D TURNER, WILLIAM A NEELY, AND W. O BARNETT

Measurement of intestinal blood flow has always been difficult to accomplish with accuracy and reproducibility Neely and Turner have devised a practical method of intermittent determination of blood flow in intestinal segments In this paper the above mentioned method of determination is used to study the effects of temporary arterial, venous and arteriovenous occlusion upon blood flow through intestinal segments

METHOD

Adult mongrel dogs were anesthetized with pentobarbital and midline abdominal incisions made A segment of ileum was selected about equidistant between the cecum and the ligament of Treitz, and the vessels to the segment were dissected free of all surrounding tissue, including nerves The vessels were sprayed with procaine throughout the procedure to prevent spasm Midway of the vascular arcade, on each side of the dissected vessel pair, the gut was severed and all bleeders tied, allowing the lumen to remain open The isolated segment was placed on an aluminum pan suspended from a Statham G 1 8 350 strain gauge transducer so that the free portions of the vessels were in a horizontal position The intestinal segment was weighed by zero suppression of the recorder before and after the occlusion periods

Control blood flow measurements were made after which the vessel or vessels were occluded by rubber covered clamps In one group the vein was occluded for 30 minutes in another the artery was occluded for 1 hour, and in still another group both the artery and the vein occluded for 1 hour Immediately following release of the occlusion, blood flow determinations were made Measurements were repeated at 1 hour, and sometimes at 2 hour intervals following release of the occlusion

The method required the dogs to remain anesthetized in one position for 3 hours or more, therefore, control determinations without vascular occlusion were done at the same time intervals as in the vascular occlusion groups Analysis of the results of this group showed no significant change in blood flow, thus permitting the assumption that significant changes in blood flow in other animals were due to the occlusion

RESULTS

The mean control blood flow in 29 dogs was 0 31 gm of blood/gm of gut tissue/min

Thirty minute occlusion of segmental vein. (*Table 1*) This group contained 9 dogs The reduction in blood flow immediately after release of the occlusion and also at 1 hour after release was significant ($P < 005$)

One hour occlusion of the artery and vein. (*Table 1*) This group contained 10 animals in which no significant change in blood flow occurred

One hour occlusion of the artery. (*Table 1*) In 10 animals blood flow was significantly decreased immediately and at 1 hour after release of occlusion ($P < 01$)

* From the Departments of Surgery and Biochemistry University of Mississippi Medical Center Jackson Supported by National Institutes of Health Research Grant Number 4574 (C2)

Table 1 Means of Blood Flow † in Intestinal Segments

BEFORE AND AFTER THIRTY MINUTE VENOUS OCCLUSION			
NUMBER OF DOGS	CONTROL	IMMEDIATELY AFTER RELEASE	ONE HOUR AFTER RELEASE
9	0 27	0 07 (P< 005)	0 13 (P< 005)
BEFORE AND AFTER ONE HOUR OCCLUSION OF ARTERY AND VEIN			
NUMBER OF DOGS	CONTROL	IMMEDIATELY AFTER RELEASE	ONE HOUR AFTER RELEASE
10	0 255	0 265 (P< 80)	0 170 (P>0 5)
BEFORE AND AFTER ARTERIAL OCCLUSION FOR ONE HOUR			
NUMBER OF DOGS	CONTROL	IMMEDIATELY AFTER RELEASE	ONE HOUR AFTER RELEASE
10	0 389	0 209 (P< 01)	0 2[illegible]0 (P< 01)

† (cc /gm /min)

DISCUSSION

This study demonstrates that venous occlusion of the intestines is more detrimental to blood flow than is arterial or arteriovenous occlusion Thirty minute occlusion of the mesenteric vein reduced blood flow markedly The reason or reasons for this reduction are not clear, although certainly interstitial hydrostatic pressure is increased due to exudation of fluid into the interstitial spaces following increased capillary pressure

The intestines have a rather great tolerance to arteriovenous occlusion Arterial occlusion alone produced significant decreases in blood flow This *differs from results obtained from studies of renal artery* and renal arteriovenous occlusion It is noted that oxygen consumption of renal tissue is greater than that of intestinal tissue

Study of this same problem in heparinized dogs might be illuminating in view of Crowell s[1] finding that heparin prolongs survival time in dogs after temporary circulatory arrest

SUMMARY

1 The mean control blood flow in 29 intestinal segments was found to be 0 31 cc /gm tissue/min

2 Thirty minute occlusion of the venous drainage of intestinal segments caused a significant decrease in blood flow

3 A *much* less marked but *significant decrease* occurred after a 1 hour occlusion of the artery

4 One hour arteriovenous stasis caused no significant change in blood flow in the segment

REFERENCE

1 Crowell J W Sharpe G P Lambright R L and Read W I Surgery *38* 696 1955

URINARY EXCRETION PRODUCTS IN STRANGULATION OBSTRUCTION *

BENJAMIN B. JACKSON

The biochemical character of the peritoneal fluid in strangulation obstruction seems to be dependent upon the concentration of red blood cells and their degradation by trauma and bacterial and intestinal enzymes. The possibility of the detection of significant excretory products in animals undergoing strangulation appears proportional to the altered intestinal permeability, substances formed in the peritoneal cavity, capability of peritoneal absorption into the blood stream and/or lymphatics of these compounds, and their subsequent elimination in the urine by the kidney in measurable quantities. The aim of this experiment was to develop a laboratory test that would differentiate strangulation obstruction from simple mechanical obstruction and free blood in the peritoneal cavity.

METHOD

Fifty female dogs were chosen for this study. Females were selected because of the ease of catheterization. Six animals were excluded because they died from other causes. Ten categories using 5 animals in each category were set up to study strangulation obstruction: 1) simple mechanical obstruction; 2) arterial occlusion, long loop (over 6 feet); 3) arterial occlusion, short loop (1 to 3 feet); 4) venous occlusion, long loop (over 6 feet); 5) venous occlusion, short loop (1 to 3 feet); 6) closed loop obstruction (2 feet); 7) whole heparinized blood in the peritoneal cavity; 8) whole heparinized blood in the peritoneal cavity with strangulation; 9) oxyhemoglobin in the peritoneal cavity with without strangulation; 10) oxyhemoglobin in the peritoneal cavity with strangulation obstruction.

The peritoneal fluid, blood serum, and urine were studied for porphyrins, indicans and oxy and met hemoglobins. Watson's qualitative test for porphobilinogen using the Ehrlich reaction was used.[1] Fluorescence of heated, acidified urine and serum were used to detect uro and corproporphyrins.[2, 3] The Obermayer reaction was utilized to recover indicans.[4] Oxyhemoglobin was detected spectroscopically and spectrophotometrically.[4]

Character of Peritoneal Fluid (time of death). 1) Venous obstruction (long loop), high hematocrit, 500 to 1600 cc. total exudate with very few degraded red blood cells. 2) Venous obstruction (short loop), high hematocrit with later degradation to oxyhemoglobin as main constituent (3 to 5.5 gm.%), total peritoneal fluid 350 to 800 cc. 3) Arterial occlusion (long loop), low hematocrit with gradual late increase in oxyhemoglobin (1 to 1.5 gm.%), total peritoneal fluid 100 to 458 cc. 4) Arterial occlusion (short loop), similar to #3, early perforation 150 to 260 cc. peritoneal fluid, oxyhemoglobin (1 to 1.5 gm.%). 5) closed loop (1 to 2 feet). High concentration of oxyhemoglobin (2 to 6 gm.%), 238 to 1400 cc. total peritoneal exudate, 6) simple mechanical obstruction, no oxyhemoglobin, 100 to 200 cc. straw colored fluid.

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* From the Department of Surgery, University of Louisville School of Medicine. Supported in part by National Institute of Health Grant A-2160.

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* From the Department of Surgery University of Louisville School of Medicine Supported in part by National Institute of Health Grant A 2160

DISCUSSION

No porphyrins were detectable in the peritoneal fluid, serum or urine by the methods utilized in this study The abnormal hemin pigment that was recovered by Nemir *et al* [5] and Cohn *et al* [6] in the peritoneal fluid and serum of dogs undergoing strangulation and in one patient with vascular occlusion could not be demonstrated However, a pinkish orange urobilin like compound formed during the Ehrlich reaction was extracted by chloroform Watson [7] noticed this same compound in a patient with porphyria complicated by a volvulus He considered it an atypical porphobilin This porphobilin exhibited a maximum absorption band at 490 mM A similar compound was detected in the urine of a case of superior mesentery artery occlusion in an elderly patient at the Louisville General Hospital This substance disappeared 36 hours after the resection of the nonviable bowel followed by reappearance in the urine 48 hours before her demise which occurred 5 days after resection It must be stated that the material has been detected in varying amounts following laparotomy, simple mechanical obstruction, and on nonoperated controls There appears to be an increase in the quantity of this unknown material in the animals with strangulation obstruction and more prominent in those with arterial occlusion The real significance of the porphobilin remains obscure and additional work will have to be done to clarify its relationship to blood degradation in strangulation obstruction

Indicans were present in large amounts in simple mechanical obstruction normal controls and all dogs undergoing strangulation Whole heparinized blood injected into the peritoneal cavity in nonstrangulated dogs resulted in no increase in porphobilin or oxyhemoglobin in the serum or urine Oxyhemoglobin injected into the peritoneal cavity of normal dogs in concentrations as great as 5 gm /100 cc resulted in the urinary excretion of oxyhemoglobin Similarly, oxyhemoglobin injected into the peritoneal cavity of stable dogs with strangulation obstruction in concentrations as large as 5 gm % was productive of oxyhemoglobin in the serum and urine In 2 dogs with closed loop obstruction oxyhemoglobin occurred in the peritoneal fluid as early as 10 hours in amounts as great as 5 gm % It never appeared in simple mechanical obstructions arterial occlusions and 8 out of 10 dogs with venous occlusion The oxyhemoglobin must be detected in the blood serum or its presence in the urine is most likely attributable to local disturbances in the genitourinary tract In view of the fact that oxyhemoglobin in adequate quantities appeared in the urine so late, other clinical signs are apt to provide the diagnosis of strangulation before it is detectable in the urine However, red urine with oxyhemoglobin in the serum following resection and preceded by an interval of clear urine may suggest the progression of necrosis in the bowel and the consequent degradation of red blood cells in the peritoneal cavity and might logically suggest to the operator the need for a second look

SUMMARY AND CONCLUSIONS

1 No porphyrins could be detected in the peritoneal fluid blood serum or urine in animals undergoing strangulation obstruction

2 In the urine of dogs undergoing strangulation obstruction a pinkish

orange porphobilin with a maximum absorption band at 490 mM was observed in the chloroform layer of the Ehrlich reaction. This atypical porphobilin was present in some controls immediately following laparotomy, and venous occlusion but it was definitely more prominent in arterial occlusion in long loops The signficance of this material remains obscure at this time.

3 Indicans occur in large quantities in simple mechanical and strangulation obstructions and appear to have no differential significance.

4 Oxyhemoglobin is the largest constituent of the peritoneal fluid in short loop venous occlusion and closed loop obstructions after 10 to 12 hours. Long loop venous occlusion yielded unchanged blood with a high hematocrit in the peritoneal fluid. Oxyhemglobin appears in small amounts (1 to 1.5 gm %) in the peritoneal fluid of animals with arterial occlusions

5 Oxyhemoglobin can be absorbed in amounts adequate for detection in the urine in normal and stable animals undergoing strangulation if the concentration in the peritoneal cavity exceeds 5 gm/100 cc. Whole heparinized blood in the peritoneal cavity produced no detectable changes in the excretory products in the urine.

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CHOLESTEROL SYNTHESIS IN HUMAN LIVER *

C B DAVIS, JR, G E COX, C BRUCE TAYLOR, AND S L CROSS

Based partly on a single study of a single subject[1] the medical literature has for several years fostered the figure of about 2 grams per day as the rate of endogenous synthesis of cholesterol by the human body It has been generally accepted, by inference from animal experiments, that most of this synthesis occurs in the liver, which is credited with a synthetic activity of about 1 5 to 2 0 gm of cholesterol per day Animal experiments have generally demonstrated a striking suppression of hepatic cholesterol synthesis following the ingestion of dietary cholesterol, this appears to be a homeostatic mechanism providing a type of compensation for dietary cholesterol to maintain relatively constant the total amount of cholesterol entering the plasma[2] Extrahepatic tissues do not show such a homeostatic response to dietary cholesterol If human liver is capable of homeostatic suppression in response to dietary cholesterol, and if 1 5 to 2 0 gm per day closely approximates the normal maximal rate of human hepatic cholesterol synthesis, then the human should be capable of compensating for dietary cholesterol in quantities up to about 2 grams per day

These figures, however, are open to some uncertainty, *in vivo* methods may give reliable figures for total body synthesis of cholesterol, but generally such methods do not delineate the specific contribution of various tissues The relative contribution of hepatic versus extrahepatic tissues to total body cholesterol synthesis assumes importance when one realizes that only liver possesses any significant capacity to regulate its synthetic activity according to the quantity of cholesterol being absorbed from the diet Without particularly questioning the accepted figures for human total body cholesterol synthesis our laboratory has deemed it worth while to attempt to measure the rate of cholesterol synthesis in human liver, using the most specific and precise technique currently available The *in vitro* technique of incubating tissue slices with labelled (radioactive) cholesterol precursors seems to be the best at this time

METHOD

Through the cooperation of the surgical staff, we have procured 36 surgical liver biopsies from patients undergoing surgery generally for nonhepatic diseases Biopsies, 2 to 6 grams in size, were obtained fresh and immediately transported to our laboratory where a Stadie Riggs tissue slicer was used to prepare sections of 0 5 mm thickness Duplicate or triplicate samples from

* From the Departments of Surgery and Pathology, Presbyterian St Luke's Hospital Chicago Supported by the Chicago, the Illinois, and the American Heart Associations the Life Insurance Medical Research Fund the Ames Company of Elkhart Indiana the National Heart Institute National Institutes of Health (H 1630 H 3215) and the Otho S A Sprague Memorial Institute

each subject were incubated, with gentle shaking, for 3 hours in Krebs-Ringer phosphate buffer at pH 6.5 and temperature 37°C. Each sample consisted of 1.0 gm. of tissue slices in 10 ml. buffer containing 12.5 mc. of C^{14} in the form of 12.3 mg. sodium acetate. After completion of incubation, the samples were hydrolyzed and the cholesterol extracted with petroleum ether. Duplicate aliquots of the extract were taken to determine the total cholesterol concentration according to the method of Sperry and Webb.[3] The cholesterol in the remaining extract was precipitated with digitonin; the precipitate was washed thoroughly, dried, and radioassayed with a thin-window Geiger counter. The percentage uptake of the added C^{14} acetate into cholesterol was calculated, and results expressed as milligrams of cholesterol synthesized (from exogenous labelled acetate) per 100 gm. of liver per hour. Whenever sufficient tissue was available, portions were extracted in Soxhlet apparati, and total cholesterol analyses performed as a continuing check on the above technique.

RESULTS

Figure 1 depicts the results to date of our estimation of the rate of cholesterol synthesis in human liver slices. On the vertical axis each block represents a single case. The horizontal axis plots the rate of cholesterol synthesis (from added acetate) in units of mg. cholesterol synthesized/100 gm. liver/hour. The values have ranged from 0.01 to 1.10, with a mean of 0.32 and a mode of 0 30. Calculations show that at a synthetic rate of 0.30 mg./100 gm./hour, a 1500 gm liver throughout 24 hours would synthesize a total of 108 mg. of cholesterol. The maximum rate of 1.1 corresponds to a total 24 hour hepatic cholesterol synthesis of 396 mg.

Tabulation of the total cholesterol concentration of the liver biopsies, supplemented with analyses from several random autopsy liver specimens, showed that, in general, the average concentration of total cholesterol in the liver of unselected humans (values clustering around 325 mg. %) appears about ⅓ higher than levels in control low-cholesterol fed dogs, monkeys and rats (with values clustering around 250 mg. %).

Serum cholesterol values are not presented because many blood samples could not be taken until just prior to or following surgery, and stress conditions are known to produce significant fluctuations (increases or decreases) in blood levels of cholesterol. It has not been demonstrated, and it is doubted, that such rapid changes in the total cholesterol level or cholesterol synthesis rates of liver are associated with stress.

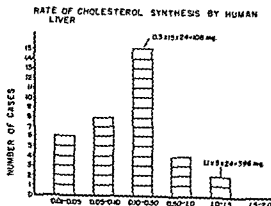


Fig 1 Rate of synthesis (calculated from the uptake of labelled acetate) of cholesterol in surgical biopsies of human liver.

DISCUSSION

The rate of cholesterol synthesis in human liver by our estimation is many fold lower than is generally accepted by medical personnel today, i.e., 0.1 to 0.4 gm. as compared to 2.0 gm. Several possible interpretations should be considered: (a) previous values are erroneously high; (b) our values are erroneously low; (c) both values are reasonably accurate, but ours represent specifically the contribution of liver tissue, whereas previous values also include the contribution of extrahepatic tissues, which would therefore appear to be by far the most important endogenous source of plasma cholesterol.

We have no definite evidence to support (a) above. However, to rule out the possibility of (b), the rate of cholesterol synthesis in rat liver as estimated by us was compared with the rates of hepatic cholesterol turnover and bile acid synthesis as determined by other investigators using entirely different procedures. Our values and their values were surprisingly close. At present, then, we accept (c) as the most likely interpretation.

Additional support for (c) has recently been obtained from *in vivo* experiments.[4] Evidence for the preeminence of liver as a source of plasma cholesterol in low cholesterol fed dogs includes the striking suppression of acetate uptake (into plasma cholesterol) following ingestion of dietary cholesterol. This phenomenon is very pronounced in dogs. The amount of acetate uptake present after cholesterol feeding apparently represents the activity of extrahepatic tissues; the quantitative difference between the control acetate uptake curve and the high cholesterol acetate uptake curve apparently represents the activity of the liver which is suppressed by cholesterol feeding. In dogs, by this procedure, more than 90% of the control acetate uptake (into plasma cholesterol) appears to occur in the liver; this has been interpreted as indicating that more than 90% of plasma cholesterol in low cholesterol fed dogs is synthesized in the liver.

This same technique was recently applied to humans to estimate the maximal percentage of plasma cholesterol that may be synthesized by the liver. Four subjects ingested a very low cholesterol diet for several weeks and then each received 100 mc. of C-14 sodium acetate orally. Blood samples were taken and the percentage uptake of the administered acetate into plasma cholesterol determined. No subject showed such striking suppression of acetate uptake as observed in dogs. One subject showed about 20% suppression, but this is much different from the 90 to 98% suppression seen in dogs; the other 3 subjects showed either no definite suppression or an actual increase in the uptake of acetate into plasma cholesterol following cholesterol ingestion.

These findings are difficult to understand, but certainly they appear to contradict the concept that plasma cholesterol in the human is synthesized largely in the liver. Thus our *in vitro* and *in vivo* data support and complement each other to the single conclusion that whatever may be the rate of total body cholesterol synthesis in man, a large part of plasma cholesterol must be synthesized in extrahepatic tissues with a much smaller contribution from the liver than previously believed. If this be true, then the human would appear to have little compensation for dietary cholesterol by means of suppression of endogenous synthesis, and the cholesterol ingested daily by many people may be much more than the amount for which they can effectively compensate. Hypercholesterolemia so prevalent in the United States may then be explain

able for many people at least on the simple basis of dietary overload of cholesterol

The levels of total cholesterol in the liver biopsies are felt to represent slight to moderate elevation and this is interpreted as entirely consistent with a general dietary overload of cholesterol

SUMMARY

1 *In vitro* studies on surgical biopsies of human liver indicate a low rate of cholesterol synthesis (from added labelled acetate) with a mean of about 0 1 gm per entire liver per 24 hours and a maximum of 0 4 gm

2 *In vivo* studies on the uptake of carbon 14 sodium acetate into the plasma cholesterol of humans failed to show the suppression after cholesterol feeding which is such a striking feature of cholesterol metabolism in dogs

3 Both studies are consistent with the hypothesis that in man the liver supplies a relatively small part of plasma cholesterol with the extrahepatic tissues being a much more important source than is currently generally believed

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STUDIES OF HEPATIC PHYSIOLOGY IN THE ISOLATED PERFUSED CALF LIVER *

ANDREW J MARTINIS PATRICK D GOLDSWORTHY THOMAS W JONES LLOYD M NYHUS ROBERT V DEVITO WADE VOLWILER AND HENRY N HARKINS

In experimental studies designed to clarify the physiology and metabolism of various organs in the intact individual one is constantly hampered by the variables introduced by other organ systems The ideal condition for these investigations might be the completely isolated organ nourished by its own blood which is supplied by a mechanical pump and oxygenator The purpose of this paper is to present a method by which the liver from a major animal can be completely isolated and maintained in a near physiologic state while being perfused mechanically with autogenous whole blood

METHOD

A bubble type oxygenator and reservoir were combined into one unit with a capacity of about 1000 cc A pair of sigmamotor finger pumps were used to

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Supported in part by U S Public Health Service grant in aid Number A1547 and Initiative 171 State of Washington

DISCUSSION

The rate of cholesterol synthesis in human liver, by our estimation, is many fold lower than is generally accepted by medical personnel today, i.e., 0.1 to 0.4 gm. as compared to 2.0 gm. Several possible interpretations should be considered: (a) previous values are erroneously high; (b) our values are erroneously low; (c) both values are reasonably accurate, but ours represent specifically the contribution of liver tissue, whereas previous values also include the contribution of extrahepatic tissues, which would therefore appear to be by far the most important endogenous source of plasma cholesterol.

We have no definite evidence to support (a) above. However, to rule out the possibility of (b), the rate of cholesterol synthesis in rat liver, as estimated by us, was compared with the rates of hepatic cholesterol turnover and bile acid synthesis as determined by other investigators using entirely different procedures. Our values and their values were surprisingly close. At present, then, we accept (c) as the most likely interpretation.

Additional support for (c) has recently been obtained from *in vivo* experiments.[4] Evidence for the preeminence of liver as a source of plasma cholesterol in low cholesterol fed dogs includes the striking suppression of acetate uptake (into plasma cholesterol) following ingestion of dietary cholesterol. This phenomenon is very pronounced in dogs. The amount of acetate uptake present after cholesterol feeding apparently represents the activity of extrahepatic tissues; the quantitative difference between the control acetate uptake curve and the high cholesterol acetate uptake curve apparently represents the activity of the liver which is suppressed by cholesterol feeding. In dogs, by this procedure, more than 90% of the control acetate uptake (into plasma cholesterol) appears to occur in the liver; this has been interpreted as indicating that more than 90% of plasma cholesterol in low cholesterol fed dogs is synthesized in the liver.

This same technique was recently applied to humans to estimate the maximal percentage of plasma cholesterol that may be synthesized by the liver. Four subjects ingested a very low cholesterol diet for several weeks and then each received 100 mc. of C^{14} sodium acetate orally. Blood samples were taken and the percentage uptake of the administered acetate into plasma cholesterol determined. No subject showed such striking suppression of acetate uptake as observed in dogs. One subject showed about 20% suppression, but this is much different from the 90 to 98% suppression seen in dogs; the other 3 subjects showed either no definite suppression or an actual increase in the uptake of acetate into plasma cholesterol following cholesterol ingestion.

These findings are difficult to understand, but certainly they appear to contradict the concept that plasma cholesterol in the human is synthesized largely in the liver. Thus, our *in vitro* and *in vivo* data support and complement each other to the single conclusion that whatever may be the rate of total body cholesterol synthesis in man, a large part of plasma cholesterol must be synthesized in extrahepatic tissues with a much smaller contribution from the liver than previously believed. If this be true, then the human would appear to have little compensation for dietary cholesterol by means of suppression of endogenous synthesis, and the cholesterol ingested daily by many people may be much more than the amount for which they can effectively compensate. Hypercholesterolemia, so prevalent in the United States, may then be explain-

able for many people at least on the simple basis of dietary overload of cholesterol

The levels of total cholesterol in the liver biopsies are felt to represent slight to moderate elevation and this is interpreted as entirely consistent with a general dietary overload of cholesterol

SUMMARY

1 *In vitro* studies on surgical biopsies of human liver indicate a low rate of cholesterol synthesis (from added labelled acetate) with a mean of about 0 1 gm per entire liver per 24 hours and a maximum of 0 4 gm

2 *In vivo* studies on the uptake of carbon 14 sodium acetate into the plasma cholesterol of humans failed to show the suppression, after cholesterol feeding which is such a striking feature of cholesterol metabolism in dogs

3 Both studies are consistent with the hypothesis that in man the liver supplies a relatively small part of plasma cholesterol with the extrahepatic tissues being a much more important source than is currently generally believed

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perfuse the hepatic artery and the portal vein. The hepatic venous blood was collected in a small funnel and allowed to flow back to the oxygenator. The entire apparatus except the pumps and the manometers was enclosed in a wood and plastic box. The box was lined with heating elements and a humidifier to maintain the liver at body temperature and moist (Fig. 1).

Young bull calves were used in these experiments because of their ready availability in a dairy area, and because they are of suitable size to allow completion of the hepatectomy and still provide enough blood to fill the oxygenator and reservoir. The calves varied in size from 20 to 40 kg. and were from 1 to 4 weeks of age. Under intravenous pentobarbital sodium anesthesia the liver was approached through a midline thoracoabdominal incision The common bile duct was cannulated and the cystic duct was ligated. The hepatic artery and the portal vein were isolated and all diaphragmatic and lumbar attachments of the liver were freed. The animal was heparinized and the aorta was cannulated below the renal arteries. The oxygenator and the reservoir were filled with blood from this aortic cannula. When all air was removed from the perfusing system, the portal vein and then the hepatic artery were rapidly cannulated, preventing liver hypoxia. The vena cava below the liver was clamped and divided. A Potts clamp was placed high on the thoracic inferior vena cava, and the vessel was divided above the clamp. The liver was then quickly transferred to the box and positioned on a gauze sling with the cut vena cava hanging free in a funnel. The Potts clamp was removed and the blood was allowed to run into the funnel. The average time of transfer (complete vena cava occlusion) was 20 seconds. Flow rates were adjusted to maintain a portal vein pressure of 12 to 20 cm. of blood and a hepatic artery pressure of 10 to 15 cm. of Hg. Arterial blood samples were drawn from stopcocks ahead of the pumps, and venous blood was taken from stopcocks on the venous return tubing.

In order to assess objectively the level of performance of the isolated preparation, comparative studies were performed on intact but anesthetized, operated, and heparinized animals Blood samples were taken from the portal

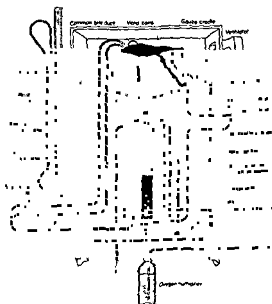


Fig 1 Schematic representation of the perfusion apparatus

vein by threading a catheter through a mesenteric vein to the porta hepatis. Peripheral venous samples were taken from the femoral vein and arterial samples were obtained from the femoral artery. Hepatic vein samples were obtained from the intrahepatic vena cava; the systemic vena caval blood having been shunted around the liver. The hepatic vein flow was connected to the vena caval bypass by means of a T-tube. In this way the sampling of hepatic outflow as well as the measurement of hepatic blood flow was done merely by diverting the flow into a suitable container. Portal vein and hepatic artery flows were measured by alternate occlusion of the vessels. Bile flow was measured as in the isolated preparation.

RESULTS

Preparations indicating good metabolic activity and having an excellent gross appearance have run as long as 12 hours. At the end of this time either the blood supply had become depleted or the liver had taken on a congested, edematous appearance. The usual duration of each experiment was about 6 hours.

Blood Flow. In order to maintain a fairly constant range of inflow pressures it was necessary to adjust the pump output to about one half of that which was found in the intact preparation. Studies in the intact animal revealed a total hepatic flow of about 1 cc./min./gm. of liver tissue. In the isolated preparation total flows ranged from 85 to 380 cc./min. but the usual value varied from 200 to 380 cc./min. or about 0.60 cc./min./gm. of liver tissue. Hepatic artery flow has varied from 46 to 180 cc./min. but usually 80 to 90 cc./min., constituting about ⅓ of the total flow.

Oxygen Utilization. Oxygen utilization has been a difficult entity to evaluate in the intact animal. By measuring the rate and oxygen content of the hepatic inflow and outflow the oxygen uptake by the intact liver was determined. A value of 0 034 cc./min./gm. of liver tissue was obtained. In the isolated preparation the oxygen consumption was somewhat lower, the range being from 0.015 to 0.027 cc./min./gm. of liver tissue. There was no direct correlation between rate of flow and oxygen uptake. The utilization appeared to remain at a constant level in spite of an increased availability of oxygen.

Bile Production. Following complete isolation of the liver there was an immediate decrease in the rate of bile production which persisted for the extent of the experiment. Studies on the intact specimen and on the flow rates before isolation showed a rate of bile excretion ranging from 15 to 38 cc./hour. Values in the isolated specimen have varied from 1.3 to 9.66 cc./hour, and the rate often fluctuated during the course of each individual experiment.

BSP Clearance. Bromsulfalein clearance studies were performed in both the intact and the isolated preparations. In the intact specimen the dye was administered directly into the vena cava following the dosage schedule of 10 mg./kg. of body weight. Blood samples were subsequently withdrawn from the cava distal to the injection site. In the isolated specimen 50 mg. of the bromsulfalein were injected into the oxygenator and the collection was made from the venous side. Figure 2 shows a representative pair of curves, one from the isolated and one from the intact preparation, illustrating the striking similarity in the patterns of BSP clearance from the blood. Figure 3 is a representative pair of curves from the same preparations showing the pattern of the appearance of BSP in the bile.

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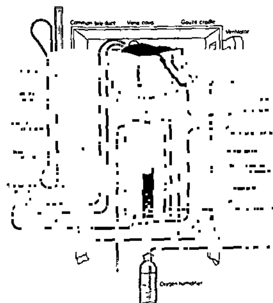


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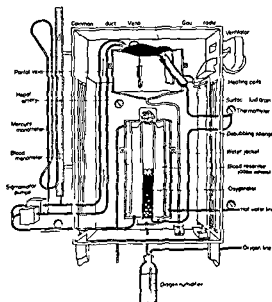


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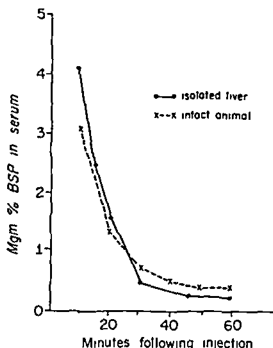


Fig 2 Blood BSP levels following injection of the dye (Representative pair of curves one from the isolated liver and one from the intact preparation)

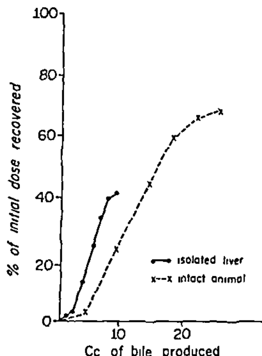


Fig 3 Pattern of recovery of BSP in the bile (Representative pair of curves one from the isolated liver and one from the intact animal)

DISCUSSION

Rate of blood flow in the intact liver appears to be fairly constant in most species Our values for both blood flow and oxygen utilization in the calf correspond well to the values reported by Blalock and Mason[1] in the dog

The decreased blood flow seen in the isolated liver is probably a function of changes in vasomotor tone secondary to denervation and lack of circulating vasotonins The decreased oxygen uptake is a process which might be anticipated when an organ is removed from its normal environment and not called upon to perform all of its physiologic functions The lack of correlation between rate of flow and oxygen uptake was a finding similar to that of Fisher and his group[2] in experiments on the totally arterialized liver of the dog

The similarity between the BSP excretion patterns in the isolated and the intact preparations would appear to indicate that gross changes in hepatic function have not occurred Andrews[3] in his studies on semi isolated dog livers, found that biliary excretion of the dye occurred from the hepatic arterial bed alone and suggested that biliary function was dependent upon arterial flow This was not the case in the isolated calf liver Bile production was not significantly different when the hepatic artery was absent from the perfusion, and biliary excretion of the dye was prompt and rapid despite the exclusion of the hepatic arterial supply

We have performed over 10 experiments with an isolated preparation Our initial attempts met with rather infrequent success but now, as our techniques have improved, we routinely obtain preparations showing good color, texture, and function, lasting many hours

SUMMARY

1. A method by which the liver from the calf can be isolated and perfused mechanically for prolonged periods of time with autogenous blood is presented.

2. Preliminary data indicate good, continuing function of the organ.

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EFFECT OF SEVERE BURN ON LIVER REGENERATION *

STANLEY M. LEVENSON, LEO V. CROWLEY,
JAMES F. OATES AND ANDRE D. GLINOS

During the past quarter century, there have appeared innumerable reports of biochemical processes that are changed by injury. Apparently, almost all metabolites are so affected but there is little objective information of the physiologic and clinical consequences of these early metabolic changes. As part of a continuing investigation of these problems, our laboratory has previously reported studies of laparotomy wounds in seriously injured animals.[1,2] We are here reporting the effect of severe burns on liver regeneration after partial hepatectomy.

METHOD AND RESULTS

Experiment 1. Twenty four male albino rats, weighing about 230 to 250 gm., Walter Reed Army Institute of Research strain, were fed a commercial † rat chow *ad libitum* prior to operation. Partial hepatectomies (70%) were performed in all rats under light ether anesthesia.[3] Half the animals were then burned by dipping their unclipped backs in hot water (73° C. for 30 sec.). This resulted in 3rd degree burns involving 30 to 35% of the body surface. The other rats were not burned. Postoperatively, all animals were fed by tube 10 ml. of 5% glucose in 0.9% saline 6 hours after operation and then every 12 hours. Forty-eight hours after operation, the animals were sacrificed under light ether anesthesia, cardiac punctures were done and as much blood removed by aspiration as possible. The abdominal organs were then eviscerated, the liver excised, and excess blood blotted.

The burned animals weighed more than their controls at sacrifice due to the edema in the burned area. The serum total protein and albumin concentrations of the burned animals were lower than those of the controls, 10%

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† D & G Rat and Mouse Baked Biscuits. Dietrich and Gambrill, Inc. Frederick, Maryland.

* From the Department of Surgical Metabolism, Division of Surgery, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

and 25% respectively. Each of these differences was statistically significant ($p = 0.02$). There were no significant differences between the two groups in any of the other parameters measured. These included liver weight, wet and dry; liver protein nitrogen concentration and content; and mitotic counts (Table 1).

Experiment 2. In view of the fact that a 70% hepatectomy induces what well may be a maximal rate of regeneration, the possible accelerating effect of a severe thermal burn of the skin on liver regeneration might not be demonstrable in animals with hepatectomies of this extent. Accordingly a similar experiment was done, except that 35% hepatectomies (removal of median lobe) rather than 70% hepatectomies were done. Serum electrophoretic analyses showed a marked depression of albumin concentration (45% lower than normal) and a striking elevation of the α_2 globulin (twice normal) in the burned animal. The average weight and protein content (but not concentration) of the regenerated liver of the burned group, 48 hours postoperatively, were significantly higher than those of the unburned rats (Table 2). Mitotic counts have not yet been done.

Experiment 3. As noted in Table 3, the relative rates of liver regeneration following the 70% hepatectomies were significantly greater than following the 35% hepatectomies. In fact, no increase in liver weight over that calculated left at operation had occurred in the 48 hours following the 35% hepatectomies. It will be recalled that these observations were made in animals receiving only 5% glucose in saline postoperatively. Accordingly, in another experiment we fed, by tube, a nutritionally "complete" rat diet (Table 4) beginning 24 hours after operation. Thirty-eight Walter Reed pathogen free male albino rats, weighing about 275 to 295 gm. were given an *ad libitum* diet of commercial rat chow and water up to the time of operation. Median lobe hepatectomies (35%) were performed in all the rats under light ether anesthesia; 17 rats were burned immediately afterwards by dipping their backs in hot water (73°C., for 30 sec.). On the afternoon of the hepatectomy, all rats were given 10 cc. of glucose and saline by pharyngeal tube and thereafter, until sacrificed, each was given 10 cc. of the liquid diet twice daily by tube.

Liver regeneration (weight and protein content) was now apparent in both the burned and unburned animals; it was significantly greater in the burned animals (Table 5). The plasma protein analyses and mitotic counts are not yet completed.

DISCUSSION

Levenson, Braasch, Mueller and Crowley[4] have previously noted a striking difference between the turnover rates of liver protein and carcass protein in severely burned rats. This difference was established by the use of N^{15} labelled glycine. While liver protein turned over very rapidly, the total protein content of the liver remained normal or, at times, increased. On the other hand, the carcass protein turned over slowly, but it gradually decreased. The amount of nitrogen lost from the carcass was equal *to the extraurinary nitrogen* excreted by the burned rats. Levenson, Pirani, Braasch and Waterman[1] also noted a significant delay of healing of laparotomy wounds in burned rats. The N^{15} data suggested to us that healing of liver "wounds" in severely burned rats should not be slower, but, perhaps, faster than normal. This hypothesis was also suggested by experiments of Glinos[5] indicating that depletion of plasma proteins through plasmapheresis initiates active growth

Table 1 Effect of Severe Burn on Liver Regeneration Following 70% Hepatectomy (Rats)

	INITIAL			at 18*		
	CONTROL	BURNED	P VALUE	CONTROL	BURNED	P VALUE
No. of Animals	12	12	—	12	12	—
Body Weight, gm	238±8 †	239±9	.1	205±15	218±15	.05
Liver Removed, gm	6.28±0.83	6.21±0.95	.5	4.10±0.15	4.42±0.51	.1
Liver H_2O, %	70.1±1.0	70.5±0.9	.1	70.2±1.1	70.9±1.8	.3
Liver Prot. N Conc., mg %	3.27±0.41	3.01±0.13	.1	3.09±.25	3.03±0.14	.5
Total Liver Prot. N, mg	—	—	—	126±11	136±17	.1
SERUM PROTEINS* gm/100 ml						
alb				2.17±.11	1.64±.58	0.02
α_1				0.91	0.93	
α_2				0.68±.17	0.83±.16	0.05
β				1.08±.13	1.14±.13	0.3
γ				0.95±.29	0.75±.18	0.05
Total				5.8±.38	5.28±.53	0.02
Alb/Glob				0.79	0.16	

* Total protein determined by a biuret method; the separate proteins were determined by paper electrophoresis
† Mean ± standard deviation

Table 2 Effect of Severe Burn on Liver Regeneration Following 35% Hepatectomy (Rats)

	INITIAL			at 48*		
	CONTROL	BURNED	T VALUE	CONTROL	BURNED	P VALUE
No of Animals	12	9	—	12	9	—
Body Weight gm	290±7	292±8 †	5	243±8	253±10	02
Liver Removed gm	3 38± 33	3 53± 16	4	5 20± 33	6 02± 37	001
Liver H O %	71 2±1 3	71 3±2 1	8	69 1±1 6	70 8±2 1	05
Liver Prot N Conc mg %	3 12± 36	3 29± 39	3	3 39± 30	3 39± 17	1 0
Total Liver Prot N mg	301±50	332±57	2	176±15	204±9	001
SERUM PROTEINS* gm/100 ml						
alb				3 36± 17	1 88± 17	01
a_1				1 20	1 22	
a_2				0 49± 08	0 97± 15	02
β				0 77± 06	0 91± 06	05
γ				0 33	0 28	
Total				6 06± 22	5 29± 17	02
Alb/glob				1 25	0 55	

* Total protein determined by a biuret method The separate proteins were determined by paper electrophoresis

† Mean ± standard deviation

Table 3. Effect of Size of Hepatectomy on Liver Regeneration †

	GRAMS	
70% HEPATECTOMY	CONTROL	BURNED
Residual †† liver wt. at operation	2.7	2.7
Liver wt. 48° postop	4.1	4.4
33% HEPATECTOMY		
Residual †† liver wt. at operation	6.3	6.6
Liver wt. 48° postop	5.2	6.0

† glucose & saline postop

†† calculated as follows

$$\text{wt. of residual liver at operation} = \frac{\text{wt. of liver removed}}{\%\ \text{Hepatectomy}} - \text{wt. of liver removed}$$

of the intact liver, it may be recalled that hypoproteinemia and hypoalbuminemia are characteristic of severely burned animals. Our present results support our hypothesis.

Cuthbertson[6] suggested some years ago from teleological considerations "that the general reaction by which labile protein is catabolized as a result of injury may serve to provide energy or amino acids, or both, for the healing process and that this is a primitive response independent of food, for a wounded animal is necessarily reduced in its capacity to feed itself." Our

Table 4. Composition of Liquid Rat Diet

Sucrose	860 gm
Egg albumin	160 gm
Cellu flour	120 gm
Dried brewers yeast	100 gm
Cod liver oil	30 gm
Wheat germ oil	30 gm
Mazola oil	30 gm

SALT MIXTURE		VITAMIN MIXTURE	
Sodium phosphate monobasic	45 gm	Choline	2.7 gm
Potassium phosphate monobasic	5 gm	Niacin	50 mg
Calcium chloride	12 gm	Pantothenic acid	30 mg
Magnesium sulfate	1.7 gm	Pyridoxine gp	12 mg
Copper sulfate	150 mg	Riboflavin	10 mg
Ferric citrate	889 mg	Thiamin	7 mg
Manganese sulfate	717 mg	Biotin	400 μg
Zinc Chloride	25 mg	Folic acid gp	300 μg
Sodium fluoride	22 mg		
Potassium iodide	2 mg		

Made up to 2000 ml. with tap water and mixed thoroughly

Table 5 Effect of Severe Burn on Liver Regeneration Following 35% Hepatectomy (Tube-fed Rats)

	INITIAL			at 48 °		
	CONTROL	BURNED	P VALUE	CONTROL	BURNED	P VALUE
No of animals	21	17	—	21	17	—
Wt of liver removed gm	3 65± 44 †	3 84± 44	2	7 51±.53	8 37± 50	001
Liver H_2O %	70 2± 69	69 8± 75	2	69 9± 8	70 8± 9	01
Wt of dry liver removed, gm	—	—		2.26± 57	2 44± 17	01
TN of wet liver $\frac{mg}{100\ mg}$	3 10± 12	3 14± 12	.2	3 30± 25	3 14± 19	05
TPN of wet liver, $\frac{mg}{100\ mg}$	2 88± 18	2 92± 15	2	3 06± 26	2 90± 16	05
TN of liver removed mg	—	—		247±19	262±17	02
TPN of liver removed, mg	—	—		230±18	243±19	05

† Mean ± standard deviation

earlier observations of delayed healing of laparotomy wounds in burned animals do not lend any certain support to this thesis, but our present observations of faster liver regeneration in severely burned rats are in keeping with Cuthbertson's viewpoint.

The serum protein data show that the levels of total protein and albumin were lower and of α 2 globulin higher in the burned rats. In the 35% hepatectomy groups, these differences were much more pronounced than in the 70% hepatectomy groups. The changes in serum proteins which follow partial hepatectomy are proportional to the extent of the hepatectomy; where the hepatectomy is large, the changes are of such magnitude that the further modification by the burn is clearly less.

Glinos[5] has recently presented in detail a discussion of the findings which have led to his formulation of the following concepts regarding liver growth and regeneration a) growth and regeneration of the liver is controlled by chemical factors present in the circulating plasma and the extracellular fluid of the liver. b) The physiological mode of action of these factors is inhibitory. Decrease of their concentration in the plasma and the extracellular fluid results in increased growth of the liver. c) The site of synthesis of these factors is the liver itself. Partial removal of the organ leads to lowering of the extracellular concentration of the factors and this initiates growth. Regeneration and restoration of the liver to its normal size results in an increase of the extracellular concentration of the factors and this in turn inhibits further growth. d) The existing evidence strongly suggests that the factors controlling the growth of the liver are the plasma proteins.

An important consequence of these concepts, especially from the clinical standpoint, is that certain (but not all) pathological conditions characterized by hypoproteinemia and hypoalbuminemia would be expected to stimulate liver growth. Findings consistent with this view were obtained in the present study.

SUMMARY

We studied the rate of liver regeneration in pair fed normal and burned rats under a variety of conditions. Measurements of liver weights, water and protein contents, mitoses and serum protein concentrations were made.

When 70% hepatectomies were done, regeneration in the first 48 hours was very rapid, and equal, in both burned and unburned rats. Since a hepatectomy of this extent induces what may be a maximal rate of regeneration, rats subjected to 35% hepatectomies were studied. In these instances, the total liver weights and protein contents were higher in the burned animals than in their unburned controls. Liver growth in the first 48 hours was not observed in those animals fed only glucose and saline postoperatively, but was noted in rats fed a nutritionally complete liquid diet by tube.

The changes in serum protein concentrations (lowering of albumin and elevation of α 2 globulin) which followed partial hepatectomy were proportional to the extent of the hepatectomy; when the hepatectomy was 35%, these changes were strikingly accentuated in the rats also burned; when the hepatectomy was 70%, the changes were of such magnitude that the further modification by the burn was strikingly less.

The relation of these findings to current concepts of the metabolic reaction to injury and of the controlling factors of liver growth and regeneration is discussed.

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STUDIES OF LIVER FUNCTION FOLLOWING PARTIAL BILIARY OBSTRUCTION IN THE DOG *

FREDERICK G MURPHY, STANLEY C SKORYNA, AND D R WEBSTER

Impairment of biliary outflow can be complete or incomplete, the millstone of many a surgeon has been the latter The various etiological factors cannot be discussed within the scope of this presentation The management of such cases is most difficult, as the clinical manifestations are diverse [1] Some of the effects of biliary obstruction have been studied recently [2, 3, 4] MacGregor [5] has studied the effects of complete obstruction in the dog It was felt in this laboratory that an attempt should be made to follow in a serial fashion the changes which occur in dogs under experimental conditions of partial biliary obstruction, with particular reference to the effects of cholecystectomy

METHOD

Adult, healthy mongrel dogs, weighing between 18 and 52 pounds, were used in our experiments and were divided into two groups In dogs of Group 1, a ligature of medium cotton was secured over the terminal portion of the common bile duct, and a steel probe 2 mm in diameter, which had been approximated to the outside of the duct wall The probe was gently slipped out, to evoke as little trauma as possible Care was taken to place the ligature below the last hepatic duct entering the common channel In the Group 2 animals, in addition to the above procedure, a cholecystectomy was also performed with drainage of the liver bed for 3 days Penicillin and streptomycin were applied locally in the subcutaneous portion of the wound All operations were carried out under sodium nembutal anesthesia

Serum bilirubin (direct and indirect),[6] serum cholesterol (free and total) [7] alkaline phosphatase [8] total serum proteins, albumin and globulin fractions [9, 10] N P N,[11] were determined according to the references indicated

* From the Department of Experimental Surgery McGill University Montreal Supported by a grant in aid of the National Research Council of Canada

Preoperative determinations were done on all animals in a fasting state, to provide a normal range for our laboratory. Postoperatively, weekly determinations were carried out for the first 12 weeks and thence every fortnight throughout the duration of the experiments, until termination either by natural death or sacrifice of the animal.

Dogs were weighed weekly. Autopsy was performed routinely, except in a few cases of death where no fresh tissues could be obtained.

RESULTS

A total of 33 dogs were included in both groups, but it was necessary to exclude 9 of these animals as 4 became completely obstructed and 5 died in the very early postoperative period due to technical errors.

Since it is impossible to present in this article complete data of the remaining 24 dogs, the overall results have been summarized in Table 1. Tables 2 and 3 are representative samples of liver function studies in Group 1, whereas those of Group 2 are represented by Tables 4 and 5. (See pages 502–506, incl.)

DISCUSSION

The changes which occur following partial biliary obstruction are dependent upon both the degree and the duration of obstruction. From the foregoing tables it would seem that the presence or absence of a gallbladder had little effect on the over all changes observed, except possibly on the incidence of concrement formation, the difference is not statistically significant.

The most consistent finding was an elevation of the serum alkaline phosphatase in the early postoperative period in 22 of the 24 dogs; this elevation was evident in the first week and continued for several weeks. In most animals, however, after a period of 4 weeks the alkaline phosphatase activity dropped to values within normal range and remained so throughout the course of the experiment. This finding is rather interesting in view of the fact that a dissociation in the alkaline phosphatase and bilirubin levels in the serum is at present believed to be a most significant finding in partial biliary obstruction. It would appear, from our results, that such a dissociation is significant only for a relatively short interval following the onset of this type of impaired biliary outflow.

The total serum protein levels remained within normal limits in all dogs during the course of the experiment, however, commencing as early as the second week, an elevation of the globulin fraction in 13 of 22 dogs was observed. As the studies progressed it could be noted that in 11 of 21 dogs the albumin levels began to drop, starting as early as the fourth to fifth week, by which time the serum alkaline phosphatase levels had usually returned to normal. The two factors mentioned above eventually led to an alteration of the A/G ratio to 1/1 (in 19 of the 24 dogs) and, in some cases, to a reversal of this ratio. It seems unlikely that nutrition played any part in the protein changes, as the dogs, which in a few weeks postoperatively had regained most of their weight, at the time of death were relatively well nourished.

The serum cholesterol levels varied considerably. A majority showed an early rise in both total and free cholesterol, but within 2 weeks fell to values within the normal range. However, after 12 weeks or more, approximately one third of these animals revealed a serum cholesterol level below normal limits, which seems indicative of impaired metabolism of the liver. No

Table 1

OPERATIVE PROCEDURE	RESULTS	CALCULI	CULT	EARLY CHOLE. ELEV	LATE CHOLE DROP	ELEV ALK PHOS	A/C RATIO ALTER	HYPO ALB	ELEV GLOBULIN	DILATED DUCT	CHOLANGITIS	CHOLECYSTITIS	LIVER CHANGE
Cholecystectomized	Positive	5	3	6	3	8	8	4	6	9	3	—	6
	Negative	4	2	2	3	1	1	3	2	0	2	—	2
	† Not included	0	4	1	3	0	0	2	1	0	4	—	0
Non cholecystectomized	Positive	10	0	10	6	14	11	7	7	13	4	5	10
	Negative	4	9	3	5	1	3	7	7	1	4	4	3
	† Not included	1	6	2	4	0	1	1	1	1	7	6	2
Total	Positive	15	3	16	9	22	19	11	13	22	7	5	16
	Negative	8	11	5	8	2	4	10	9	1	6	4	6
	† Not included	1	10	3	7	0	1	2	1	1	11	6	2

† Not included All those cases which, for various reasons could not be included because of such factors as—sections not available, follow up period too short and one or other determination not reported

Table 2

DOG NUMBER 26 352
OPERATION 2 mm constriction of C B D

TIME WEEKS	WT LBS	CHOLESTEROL TOT	CHOLESTEROL FREE	ALK PHOS	PROTEINS TOTL	PROTEINS ALB	PROTEINS GLOB	BILIRUBIN TOTL	BILIRUBIN DIR	BILIRUBIN INDIR	NPN
Pre Op	28	148	—	1 9	6 6	4 0	2 6	.55	.30	.25	33
1	28	165	—	24 2	7 2	3 2	4 0	17	14	03	23
2	26	185	—	11 1	7 7	4 4	[illegible]	1 17	79	38	25
3	26	166	—	5 0	6 7	4 2	2 5	97	.50	47	33
4	28	133	42	4 1	6 1	4 1	2 0	61	33	.28	19
5	28	191	45	2 5	7 6	3 8	3 8	99	67	.32	40
6	26	181	55	1 8	6 2	3 8	3 4	31	13	18	29
7	26	138	49	2 4	7 4	4 9	2.5	0	0	0	21
8	28	153	40	0 5	6 6	3 7	2 9	.28	25	03	34
9	—	—	—	—	—	—	—	—	—	—	—
10	28	127	41	3 10	6 6	3 2	3 4	44	31	13	32
11	—	—	—	—	—	—	—	—	—	—	—
12	26	216	80	2 5	2 2	3 6	3 6	32	17	15	54
14	24	126	32	1 6	7 2	3.5	3 7	1 32	72	60	26
16	22	113	31	2 4	7 4	2 9	4 5	22	09	13	25
18	24	123	47	4 8	8 4	4.2	4.2	65	.27	.38	43
20											
22											
24											
26											
28											
30											
32											
34											

PATHOLOGICAL FINDINGS Cause of Death Terminal sacrifice
Gross Heart & Lungs —Normal Stomach —No ulcer Stool —brown Liver —Normal size color and consistency C B D —Dilated 2 x normal above stricture G B —Not remarkable Bile —Sluggish brown bile with many fine black concrements Culture —No growth

Microscopic Centrilobular degeneration and necrosis Many lipophages in liver No fibrosis or bile duct proliferation
C B D & G B —Changes of chronic cholecystitis and cholangitis
Lymph Nodes (taken near C B D) —Mild lipogranulomatosis reticulosa in node

DOG NUMBER 32 366
OPERATION 2 mm constriction of C B D

Table 3

TIME	WT	CHOLESTEROL		ALK	PROTEINS			BILIRUBIN			
WEEKS	LBS	TOT	FREE	PHOS	TOTL	ALB	GLOB	TOTL	DIR	INDIR	NPN
Pre Op	40	163	48	3 9	5 9	3 7	2 1	32	02	0	39
1	35	406	147	100	6 3	[illegible]	2 6	1 2 2	8 2	1 0	27
2	36	167	67	36.6	5 9	3 7	2 2	32	14	28	27
3	32	216	94	25 3	7 1	3 9	3 2	.52	24	.28	21
4	30	115	32	8 6	5 4	3 1	2.3	.50	29	.21	31
5	31	135	10	3 2	7 6	5 3	2 3	20	11	09	3
6	30	113	40	3 2	6 3	2 6	3 7	52	17	35	40
7	34	120	29	2 1	5 8	3 5	2 3	25	12	13	41
8	30	114	31	2 2	7 7	3 3	4 4	1 27	53	64	60
9	34	183	93	2 1	6 3	3.5	2 8	52	30	22	61
10	32	115	90	1 8	6 5	2 7	3 7	42	11	31	38
11	30	98	43	2 2	3 0	3 0	3 0	1 24	90	34	30
12	30	89	23	2 2	7 4	2 6	4 8	38	22	16	34
14	30	95	27	1 9	7 2	2 7	4 5	32	13	19	30
16	30	1[illegible]0	42	3 6	8 7	3 3	5 3	26	17	09	31
18											
20											
22											
24											
26											
28											
30											
32											
34											

PATHOLOGICAL FINDINGS Cause of Death Terminal sacrifice
Gross Heart & Lungs —Normal Stomach —No evidence of ulcer Stool —Normal Brown Liver —Slightly mottled but normal size and color C B D —Dilated 2 x normal above ligature G B —Not remarkable Bile —Blackish—contained many calculi up to 4 5 mm which were black Culture —No growth

Microscopic Liver —Normal appearance
C B D —Mild chronic cholangitis
G B —Chronic cholecystitis with much round cell infiltration

DOG NUMBER 13 522
OPERATION Cholecystectomy and 2 mm constriction of C B D

Table 1

TIME WEEKS	WT LBS	CHOLESTEROL TOT	CHOLESTEROL FREE	ALK PHOS	PROTEINS TOT	PROTEINS ALB	PROTEINS GLOB	BILIRUBIN TOT	BILIRUBIN DIR	BILIRUBIN INDIR	NPN
Pre Op	44	175	—	2.2	6.8	3.1	3.7	03	03	0	20
1	38	282	—	3.5	5.9	3.7	2.2	—	—	—	21
2	42	259	—	3.0	7.5	3.0	1.5	11	11	0	10
3	42	158	—	8.1	8.1	3.9	1.5	—	—	—	31
4	42	153	—	1.1	7.0	3.5	3.5	67	13	24	27
5	42	237	—	0.7	8.8	3.3	5.5	1.11	60	.51	27
6	38	111	—	1.5	7.5	2.8	1.7	.24	11	13	26
7	38	164	—	1.7	8.5	3.2	5.3	.36	14	.22	24
8	38	181	—	6.0	8.2	3.4	1.8	.26	17	09	27
9	38	149	—	1.7	8.3	3.6	1.7	.39	11	.28	24
10	42	137	—	4.1	7.6	1.0	3.7	.50	.22	28	16
11	—	—	—	—	—	—	—	—	—	—	—
12	36	169	16	4.5	8.1	3.2	1.8	.42	11	31	27
14	40	87	21	7.2	9.0	3.3	5.7	.71	.27	11	24
16	40	124	26	3.3	7.8	3.2	1.6	1.89	.78	1.10	19
18	40	106	22	2.9	6.0	1.2	1.8	1.30	17	83	36
20	40	213	85	2.5	6.7	1.3	2.1	0	0	0	30
22	40	98	26	2.7	6.7	2.9	3.8	.28	17	.31	25
24	38	82	21	1.1	6.9	2.9	1.0	.15	32	13	28
26	40	96	29	3.5	7.3	2.7	1.6	.31	.21	13	31
28											
30											
32											
34											

PATHOLOGICAL FINDINGS Cause of Death Terminal sacrifice
Gross Heart & Lungs —Normal Stomach —No ulcer
Stool —Brown Liver —Grossly normal C B D —
Duct dilated 2 X normal above ligature Bile —
Golden brown with only one small black calculus
noted

Microscopic Liver — Small foci of parenchymal necrosis scattered throughout
There is a marked inflammatory reaction in the tracts and
central veins
No evidence of cirrhosis or fibrosis
C B D —Moderate degree of chronic cholangitis

Table 5

OPERATION Cholecystectomy and 2 mm constriction of C B D

TIME	WT	CHOLESTEROL		ALK	PROTEINS			BILIRUBIN			
WEEKS	LBS	TOT	FREE	PHOS	TOTL	ALB	GLOB	TOTL	DIR	INDIR	NPN
Pre Op	25	154	47	1 7	6 0	3 6	2 4	28	09	19	27
1	22	247	81	22 6	5 5	3 4	2 1	5 5	2 7	2 8	23
2	20	128	60	51 7	5 9	3 3	2 6	3 2	1 2	1 1	26
3	20	117	41	27 4	6 7	3 3	3 4	83	39	44	25
4	18	138	44	15 2	8 6	3 0	5 6	55	34	19	23
5	20	135	41	3 7	7 1	2 3	4 8	30	14	16	23
6	18	190	60	73.5	7 3	4 1	3 2	66	32	34	22
7	22	120	41	46 9	6 1	3 1	3 0	20	11	09	22
8	22	112	26	26 0	6 7	3 4	3 3	33	14	19	22
9	20	164	57	12 7	7 3	3 6	3 7	1 73	97	76	50
10	20	164	65	7 5	6.5	3 5	3 0	87	43	44	40
11	22	85	43	4 1	6 1	3 4	2 7	88	.24	64	29
12	20	86	24	2 4	6 3	2 9	3 4	51	20	31	28
14	20	107	20	4 1	6 5	2 8	3 6	17	17	0	40
16											
18											
20											
22											
24											
26											
28											
30											
32											
34											

PATHOLOGICAL FINDINGS Cause of Death Terminal sacrifice

Gross Heart and Lungs —Normal Stomach —No ulcer Stool —Brown Liver —Size slightly reduced but grossly normal C B D —Dilated about 4 x normal above stricture Bile —No calculi and bile normal golden brown Culture No growth

Microscopic Liver —Marked bile duct proliferation Bile seen in hepatic and Kupffer cells No bile plugs A moderate degree of fibrosis around bile ducts Chronic hepatic fibrosis C B D —Not remarkable

significant alteration was noted in the ratio of free to total cholesterol in this experiment.

The serum bilirubin levels in the dogs were never markedly elevated, except in a few cases during the early postoperative period; the values were followed mainly as an index as to whether the obstruction was incomplete or complete.

The NPN values were normal and thus it is probable that any marked renal damage never existed.

As stated previously, calculi were found in 15 of the 23 dogs which were examined. These calculi were small, black and soft. The largest calculus was 8 by 10 mm. in size. No detailed chemical analysis of biliary concrements was carried out, however the gross appearance of the calculi indicates that they were composed mostly of bile pigment, with little if any cholesterol.

The present studies indicate t' [illegible] .nges existed in 16 of the dogs, ranging [illegible] to a rather marked biliary cirrhosis I [illegible] icant part, as only 20% of the bile samples cultured gave a positive result

Further morphological studies are indicated to ascertain the extent of microscopic changes in the liver structure under the conditions of experimental partial obstruction of the biliary outflow.

SUMMARY AND CONCLUSIONS

1. Dogs with partial biliary obstruction produced by placing a constricting ligature around the terminal portion of the common bile duct can be maintained for experimental studies of biliary stasis for prolonged periods of time.

2 The presence or absence of the gallbladder did not influence materially the effects of partial biliary obstruction on liver function

3 Pigment calculi were observed in the majority of animals with partial biliary tract obstruction produced by this method.

4 The most constant findings with reference to liver function were: 1) elevation of the serum alkaline phosphatase in the early phase of biliary stasis; 2) alteration of the A/G ratio, due partially to early serum globulin fraction elevation, and partially to later drop in the albumin fraction.

5 Some degree of morphological changes in the liver can be expected in the majority of dogs in which the biliary outflow has been impaired.

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HOW RELIABLE ARE LIVER FUNCTION TESTS? A REAPPRAISAL IN DOGS *

JAMES P SPELL AND JAMES D HARDY

The clinician is frequently disappointed in the quality of the diagnostic assistance gained from liver function studies where it is important to differentiate medical jaundice from surgical jaundice Also the surgeon not infrequently finds at laparotomy a liver which is grossly and obviously diseased in the presence of relatively normal preoperative liver function studies Since there appears to exist a wide range in the confidence placed by different physicians in the several tests usually employed the purpose of this study was to evaluate the effects of specific lesions produced in dogs

METHOD

Thirty (30) mongrel dogs varying in weight from 12 to 25 kg were utilized They were divided into the following seven groups in which the specific *surgical lesion indicated were produced Group 1 6 dogs control group* Group 2 6 dogs ligation of common bile duct Group 3 6 dogs ligation of hepatic artery Group 4 3 dogs occlusion of hepatic artery for one hour Group 5 3 dogs partial hepatectomy *Group 6 3 dogs ligation of portal vein Group 7 3 dogs* portacaval shunt

Each group of animals was observed for a period of one week before operation and for at least two weeks postoperatively A liver battery consisting of serum bilirubin alkaline phosphatase cephalin flocculation and thymol turbidity was run preoperatively upon each animal and then repeated postoperatively at 24 hours 48 hours 72 hours 1 week and 2 weeks

RESULTS

Group 1 (6 dogs) Control Group Three dogs in the control group were subjected to simple abdominal incision through an upper right rectus incision The abdomen was then closed without further surgery The remaining three dogs in the control group were not operated upon in any manner The *liver function values of the control group showed some minor variations but* these were not marked There was no significant difference in values for the dogs which were subjected to simple abdominal incision and closure as op*posed to the dogs which underwent no surgery*

Group 2 (6 dogs) Ligation of the Common Bile Duct Ligation of the common bile duct produced a progressive and rapid rise in the alkaline phosphatase serum bilirubin and thymol turbidity over the 2 weeks of this study Two of the 6 dogs expired 5 and 7 days postoperatively and at autopsy in each there was found a ruptured gallbladder with extensive bile peritonitis Clinical jaundice did not develop in either of these animals prior to death Three other animals of this group developed clinical jaundice *(yellowing of* sclerae and mucous membranes *of* the mouth) from the seventh to tenth day postoperatively Autopsy of these animals in each instance revealed marked distention *of the gallbladder and all the larger bile passages* There was also

* From the University of Mississippi Medical Center Jackson Supported by Army Contract Number DA [illegible] MD [illegible]

evidence of bile leakage into the abdomen These 3 animals were very weak at the time they were sacrificed (15 days postoperatively) and it is thought that they would probably have survived only a short time longer

One animal in which the common duct was ligated showed no ill effects of this procedure through his liver function tests or in his general appearance It was sacrificed 21 days postoperatively and found to have an accessory bile duct which emptied into the duodenum about ½ cm distal to the main common bile duct

Group 3 (6 dogs) Ligation of the Hepatic Artery An attempt was made to interrupt the arterial blood supply to the liver by ligating not only the hepatic artery proper but also the gastroduodenal arch the right gastric artery and the right and left hepatic arteries Since it had been shown by Markowitz Rappaport and Scott [1] that this procedure results in complete interruption of arterial blood to the liver these animals were given 600 000 units of penicillin preoperatively and 600 000 units of penicillin daily for ten days postoperatively

Ligation of the hepatic artery produced only a brief rise in the alkaline phosphatase In view of the reported grave consequences associated with complete ligation of the arterial supply to the liver it is perhaps doubtful that complete interruption of arterial supply was accomplished in this group of animals However it is also possible that the penicillin which was given to the animals pre and postoperatively was responsible for the minor nature of the changes in liver functions in this group

Group 4 (3 dogs) Occlusion of Hepatic Artery for One Hour This group of animals was treated by isolation of the right and left hepatic arteries at the point of their division from the common hepatic artery and occlusion of these branches for a period of one hour These animals were also given penicillin 600 000 units preoperatively and 600 000 units daily for a period of ten days postoperatively This temporary arterial occlusion produced little change in the liver function tests These 3 dogs were sacrificed and autopsied 15 days postoperatively In each a considerable number of adhesions was seen at the site where the hepatic arteries had been occluded but the liver and gallbladder appeared entirely normal to gross examination

Group 5 (3 dogs) Partial Hepatectomy The following technique was used for performing partial hepatectomy in these three animals Under intravenous anesthesia (35 mg nembutal/kg) the abdomen was opened through a midline incision and the hepatic triad was exposed An intestinal clamp was placed across the hepatic triad for a period of 6 or 7 minutes during which the left lateral and middle lobes including the gallbladder were rapidly clamped with Kocher clamps and amputated The stumps of the lobes were ligated with 00 silk sutures Finally the intestinal clamp was removed from the hepatic triad and circulation of the liver was allowed to resume

These animals were given penicillin 600 000 units preoperatively and 600 000 units daily postoperatively for a period of 6 days In addition each animal was given ½ pint whole milk daily fortified with Karo syrup for a period of 10 days postoperatively The animals were also fed their regular diet of dog meal Each animal survived the operation without particular incident Partial hepatectomy produced a rise in alkaline phosphatase and thymol turbidity but caused a decline in bilirubin concentration

The animals were sacrificed 15 days postoperatively and in each instance

the liver stump was found to be well healed also there was hypertrophy of the right lobe of the liver There was no evidence of bile peritonitis

Group 6 (3 dogs) Ligation of Portal Vein In 3 animals simple ligation of the portal vein was carried out In each case the animal expired within 2½ hours postoperatively Autopsy revealed massive hemorrhagic congestion of all abdominal viscera The liver was markedly congested and presented a nutmeg appearance

Group 7 (3 dogs) Portacaval Shunt In these animals a side to side porta caval shunt was performed under light nembutal anesthesia These animals did surprisingly well postoperatively and they exhibited no grossly abnormal phenomena There was a mild rise in the serum alkaline phosphatase level but other liver function tests were within normal limits Each of these animals was sacrificed 15 days postoperatively and autopsy revealed the portacaval anastomosis to be patent There was no evidence of intraabdominal or hepatic abnormality as a result of this portacaval shunt

CONCLUSIONS

In this study 4 of the liver function tests commonly used in human beings have been followed in dogs which had surgical lesions of the liver bile ducts or portal system produced by artificial means It would appear that these particular liver function tests are not too accurate in following lesions which primarily affect the blood vessels supplying the liver This conclusion is made on the basis of very few or no changes in the liver function values when lesions of the hepatic artery and portal vein were simulated i e ligation of hepatic artery portacaval shunt occlusion of hepatic artery

The liver function tests employed in this study were of definite aid in fol lowing obstructive disease of the biliary system

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THE DIFFERENTIATION OF MEDICAL FROM SURGICAL JAUNDICE BY MEANS OF SERUM TRANSAMINASE AND IRON DETERMINATIONS *

FRANZ GOLDSTEIN DAVID SELIGSON AND PAUL NEMIR JR

The cause of jaundice can be correctly diagnosed by means of a thorough his tory and physical examination in 60 to 80% of patients The use of conven tional liver function tests may raise the accuracy to 90% the additional use of liver biopsy may raise it to about 95% [1] Incorrect diagnoses are made most

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often in the group of patients with intrahepatic cholestasis. These patients, while suffering from intrahepatic disease, usually manifest the conventional laboratory findings associated with extrahepatic obstructive jaundice. Since the treatment of jaundice due to intrahepatic cholestasis is medical and that of extrahepatic obstruction is surgical, the differentiation is crucial. For these reasons the search has continued for newer and better laboratory tests which might aid in the differential diagnosis of "medical" from "surgical" jaundice.

While intrahepatic cholestasis may be associated with a variety of liver disorders, the most common and diagnostically troublesome cases are due to viral hepatitis with intrahepatic cholestasis. The term "cholangiolitic hepatitis" has been applied primarily to this group of patients, although it has also been applied to patients with drug-induced cholestasis.

The present study is a critical evaluation of serum iron and serum glutamic oxalacetic transaminase (SGO-T) determinations in the differential diagnosis of jaundice. Among 100 patients studied, viral hepatitis was the cause of jaundice in 31 patients, extrahepatic obstructive jaundice was diagnosed in 37 patients, and drug-induced jaundice (due to chlorpromazine in all instances) was present in 4 patients. Among the patients with hepatitis, 8 had acute viral hepatitis of the usual clinical or "hepatocellular" variety, 11 patients presented with acute viral hepatitis of the cholestatic type ("cholangiolitic hepatitis") and 12 patients had chronic hepatitis. The diagnosis in each instance rested on firm grounds and was confirmed by biopsy, exploration or autopsy in almost all cases. Discussion will be limited to patients with acute hepatitis and patients with extrahepatic obstructive jaundice. SGO-T was determined in all patients; serum iron was measured in 15 of 19 patients with acute hepatitis and 23 of 37 patients with extrahepatic obstruction. The highest values found in each patient were recorded.

The conventional liver function tests commonly regarded as being most helpful in differentiating hepatitis from extrahepatic obstruction are the cephalin flocculation and alkaline phosphatase determination. The cephalin flocculation was markedly positive in all 8 patients with the usual type of acute viral hepatitis. Alkaline phosphatase activity ranged from 4.4 to 9.8 Bodansky units in this group. These are the expected findings in acute hepatitis. SGO-T activity in this group ranged from 1032 to 2280 units per ml., with a mean of 1726 units per ml. (normal: 8 to 40 units per ml.). Serum iron levels determined in 7 patients of this group ranged from 245 to 458 µg per 100 ml., with a mean of 312 µg per 100 ml. (normal: 50 to 150µg per 100 ml.). This group of cases presented no diagnostic difficulties clinically or on the basis of laboratory findings.

In the 11 patients with "cholangiolitic" hepatitis, i.e., viral hepatitis with prominent intrahepatic cholestasis, the cephalin flocculations were normal in all but 1 patient, the alkaline phosphatase activity ranged between 12.9 and 35.2 Bodansky units. These values are similar to those commonly seen in patients with extrahepatic obstruction. The SGO-T levels in this group ranged from 106 to 888 units with a mean of 532 units per ml. The low value of 106 units was found in a patient first seen 13 days after the onset of jaundice. The SGO-T activities in this group were lower than in patients with the usual clinical type of acute hepatitis but tended to be higher than will be shown for the group with extrahepatic obstruction. The serum iron levels ranged between 140 and 327 µg per 100 ml. with a mean of 257 µg per 100 ml.

In the 37 patients with extrahepatic obstructive jaundice cephalin flocculations were usually normal but were positive in 3 patients The alkaline phosphatase was above 10 Bodansky units in 27 below 10 units in 10 patients The SGO T activity was normal in 4 patients elevated to less than 300 units per ml in all but 2 of the remaining patients The highest SGO T level in this group of patients was 540 units per ml and was recorded in a case of carcinomatous extrahepatic obstruction with hepatic metastases and necrosis proved on biopsy The mean of the SGO T activities in this group was 118 units per ml The serum iron determined in 23 patients of this group was normal in 15 patients elevated to less than 200 μg per 100 ml in five patients and elevated above 200 μg per 100 ml in 3 patients The mean was 112 μg per 100 ml The highest iron level of 305 μg per 100 ml was found in another patient with hepatic metastases

Conventional liver function tests chiefly flocculation tests and alkaline phosphatase determinations provide fairly good discrimination between typical cases of acute hepatitis and extrahepatic obstructive jaundice They are of no value in distinguishing cholangiolitic hepatitis from extrahepatic obstruction In Figure 1, SGO T activities are compared for the 3 groups of patients discussed acute hepatitis of the usual hepatocellular variety acute cholangiolitic hepatitis and extrahepatic obstructive jaundice There was a slight overlap between the two latter groups i e cholangiolitic hepatitis and extrahepatic obstruction In Figure 2 a similar comparison is made of the serum iron determinations for the 3 groups of patients The serum iron values in the 2 groups of patients with acute hepatitis were of a similar order of magnitude The serum iron tended to be markedly elevated in patients with acute hepatitis of both the hepatocellular and cholangiolitic types it tended to be normal in patients with extrahepatic obstruction but a considerable overlap did exist Since the serum levels of SGO T and iron both under normal and abnormal circumstances are affected by a variety of unrelated factors it appeared worth while to compare the sums of both iron and SGO T (the former expressed in μg per 100 ml the latter expressed in units per ml) between the groups of patients with hepatitis and extrahepatic

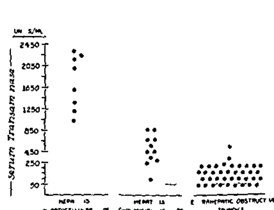


Fig 1 Comparison of serum glutamic oxalacetic transaminase (SGO T) levels between groups of patients with acute hepatitis of the hepatocellular and cholangiolitic types and with extrahepatic obstructive jaundice

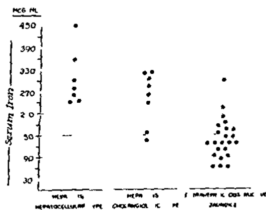


Fig 2 Comparison of serum iron levels between groups of patients with acute hepatitis of the hepatocellular" and cholangiolitic and with extrahepatic obstructive (In heading at top of Fig 2 MCG/ML should read MCG/100ML)

obstruction As was hoped, the overlap between the two groups of patients was greatly reduced (Fig. 3) The sum of serum iron and SGO-T exceeded 100 in all patients with acute hepatitis of either clinical type. In only one of 23 patients with extrahepatic obstruction in whom both SGO-T and serum iron were measured did the sum of SGO T and iron exceed 100 and this was the patient with demonstrated hepatic metastases and necrosis

DISCUSSION

The main factor determining elevations in serum levels of both iron and SGO T in jaundiced patients appears to be hepatic necrosis, excluding any coincidental extrahepatic factors Whether the slight elevations in SGO-T in extrahepatic obstruction are due to mild liver cell damage which is difficult to demonstrate histologically or due to other factors such as regurgitation of excreted transaminase, cannot be answered at this time. Hemolysis of varying degrees known to be associated with many cases of acute hepatitis[2] probably contributes to the elevations in serum iron levels in patients with hepatitis, but most of the excess iron in the serum of these patients appears to be derived from storage iron released from damaged hepatic cells In patients depleted of iron who develop hepatitis, the serum iron level may rise slightly or may rise from subnormal to normal levels. In patients with hemolytic jaundice, high serum iron levels are usually encountered in the absence of hepatic necrosis For these and other reasons it is inadvisable to depend upon serum iron levels exclusively for the differential diagnosis of jaundice.

Greatly increased activity of SGO T is probably the most consistent and most specific laboratory abnormality encountered in patients with acute viral hepatitis SGO T levels above 100 units per ml. in a jaundiced patient are very suggestive of hepatitis, viral or toxic. The chief limitation to the use of SGO T in the differential diagnosis of medical from surgical jaundice is the time factor In many cases of infectious hepatitis with a mild clinical course, the SGO T activity tends to revert to normal or near normal levels within a few days If a patient is seen late in the course of acute hepatitis, the peak of SGO T elevations may have been passed and a relatively mild and nondiagnostic elevation may be encountered which might suggest the presence of extrahepatic obstructive jaundice Since serum iron levels tend to return to

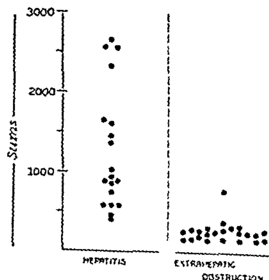


Fig 3 Comparison of the sums of serum glutamic oxalacetic transaminase expressed in units per ml and of serum iron expressed in μg per 100 ml between the groups of patients with acute viral hepatitis and with extrahepatic obstructive jaundice

normal somewhat slower than SGO T it is in these cases that serum iron determinations are especially valuable The combined use of SGO T and serum iron levels was first suggested by one of us (Seligson[3]) Even when both serum iron and SGO T values were in the borderline zone between ob structive and hepatitis jaundice the sum of both iron expressed in μg per 100 ml and of SGO T activity expressed in units per ml was 400 or higher in all patients of this series with acute hepatitis of either the hepatocellular or cholangiolitic clinical types it was below 400 in all but one of 23 patients *with obstructive jaundice Subsequent experience gives cause to additional* caution since one patient with cholangiolitic hepatitis was seen in whom the SGO T never exceeded 160 units and the iron level remained normal The patient was explored without finding extrahepatic obstruction and liver biopsy revealed very mild hepatitis Transient SGO T elevations to about 400 units per ml without concomitant serum iron elevations have been ob served in a few patients with sudden common duct obstructions These eleva tions tend to revert to normal or near normal within 24 to 48 hours The determination of SGO T and serum iron does not replace other clinical and laboratory methods of examination but represents a valuable addition to the diagnostic workup of the jaundiced patient

SUMMARY AND CONCLUSIONS

1 The diagnostic value of serum iron and SGO T was critically evaluated in a group of 100 jaundiced patients

2 The greatest value of serum iron and SGO T determinations was found to lie in the differential diagnosis of extrahepatic obstructive jaundice from cases of acute viral hepatitis with marked cholestatic features (cholangiolitic hepatitis)

3 While both serum iron and SGO T tended to be higher in the group of jaundiced patients due to cholangiolitic hepatitis than in patients with extrahepatic obstruction a significant overlap was encountered especially when patients were studied late in the course of their disease

4 The overlap between these two groups of patients was markedly reduced when serum iron and SGO T were determined simultaneously The sum of serum iron expressed in μg per 100 ml and of SGO T expressed in units per ml exceeded 400 in all 19 patients with acute hepatitis this sum was below 400 in all but one of 23 patients with extrahepatic obstructive jaundice in whom both iron and SGO T were determined

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A COMPARISON OF GALACTOSE CLEARANCE BY THE LIVER FOLLOWING PORTACAVAL TRANSPOSITION OR ECK FISTULA*

JOHN Q. OWSLEY, JR., JOHN M. GOIN, JOHN C. CLARKE, HAROLD A. HARPER AND H. J. McCORKLE

There is evidence that diminution of hepatic function occurs following portacaval shunt (Eck fistula) in dogs[1,2] but such changes do not occur in animals in which a portacaval transposition has been done[3]. The portacaval transposition procedure resembles an Eck fistula in that it diverts the portal venous blood from the liver but presumably differs from it by maintaining normal blood supply to the liver parenchyma. Therefore it appears likely that the impairment of hepatic function which occurs in dogs with Eck fistulas but not in dogs with transposition of the portal vein and vena cava may be due to the effects of ischemia on the hepatic parenchyma. In an attempt to demonstrate this a biochemical process known to be accomplished exclusively in the cellular tissues of the liver, namely the conversion of galactose to glucose[4] was compared in normal dogs, dogs in which the vena cava and portal veins had been transposed, and in dogs with Eck fistula. Recent studies of the biochemical processes by which galactose is converted to glucose have confirmed the fact that these reactions occur only in the liver[5]. When this clinical test of liver function is done galactose may be given orally or intravenously and the rate of its disappearance from the peripheral blood measured[6]. However the galactose tolerance test cannot be done in this manner for the purpose of studying liver function in dogs with Eck fistulas because the reduction in total hepatic blood flow which this operation probably produces may cause a decreased rate of removal of galactose from the peripheral blood regardless of hepatocellular functions. Therefore in order to compare the conversion of galactose to glucose by the livers of normal dogs, dogs with transposition of the vena cava and portal vein, and dogs with Eck fistulas, it was necessary to devise different surgical procedures whereby galactose could be conveyed directly to the liver in each group of experimental animals.

Three groups of adult mongrel dogs weighing between 12 and 22 kg. were selected for these experiments. Sodium pentobarbital anesthesia was given to the animals during all surgical operations and aseptic surgical methods were used. In the first group of 6 normal dogs a 16 gauge plastic (Genflex®†) catheter was placed in the portal vein just proximal to the hilus of the liver. The proximal end of the catheter was brought out through a small incision in the flank, filled with a solution of saline and heparin and occluded at its proximal end which was then implanted just beneath the skin. In this way the catheter was easily available whenever required for infusions directly into the portal vein (Fig. 1A).

Eck fistulas were made in 3 dogs. A moderately long ligated stump of proximal portal vein was left and all tributary branches of this stump were

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† Manufactured by General Cement Company, Illinois.

* From the Surgical Research Laboratories and the Department of Surgery of the University of California School of Medicine, San Francisco. Supported by a grant from the National Institute of Arthritis and Metabolic Diseases, USPHS A 1053 and the Christine Breon Fund for Medical Research.

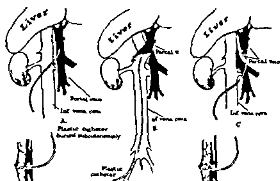


Fig 1 Diagrams indicating methods for infusing galactose directly into the portal vein of experimental animals
A Normal dog Plastic catheter has been placed in the portal vein near hilus of liver with proximal end of catheter implanted subcutaneously
B Dog with portacaval transposition Plastic catheter has been passed via femoral vein through the anastomosis between inferior vena cava and portal vein at hilus of liver
C Dog with Eck fistula Plastic catheter has been inserted into ligated stump of the portal vein and the proximal end implanted subcutaneously

ligated and divided Six to 8 weeks after this operation these animals were operated upon again at which time a 16 gauge Genflex® catheter was placed in the ligated stump of the portal vein near the hilus of the liver (Fig 1C) Patency of the portal vein stump above the ligature was proved by obtaining a free return of blood into the catheter The proximal end of the catheter was implanted in a subcutaneous position in the same manner as described for the normal dogs

Portacaval transposition was done in a third group of 4 dogs In these dogs infusions were made directly into the portal vein through a long catheter which was introduced into the inferior vena cava via the femoral vein and then passed up through the portacaval anastomosis into the portal vein near its entrance into the liver (Fig 1B)

Observations on the galactose clearance function of the liver were done on these 3 groups of animals 6 to 8 weeks after the surgical shunting procedures Solutions containing 25 gm galactose/1000 ml of distilled water were infused via the plastic catheters placed in the portal veins of the unanesthetized dogs In preliminary experiments with normal dogs galactose solution infusions were given at rates varying from 002 to 010 gm/kg/minute and peripheral venous blood samples were drawn at 15 minute intervals during the infusions The galactose content of each blood sample was measured by the method described by Althausen *et al*[7] In these experiments it was found that during infusion rates of 004 to 006 gm/kg/minute there was only a moderate rise in the peripheral blood galactose With more rapid rates of infusion a marked increase in the peripheral blood galactose occurred suggesting that the capacity of the liver to convert galactose to glucose was exceeded (Fig 2)

In an attempt to establish the maximal rate of hepatic utilization of galactose more accurately the same concentration of galactose solution was infused directly into the portal vein in normal dogs in two series of experiments one at a rate of 004 gm/kg/minute and the second at 006 gm/kg/minute The results of these experiments in a normal dog are illustrated (in Fig 3) and from them it is apparent that 004 gm/kg/minute is approximately the rate at which the normal dog liver can utilize this concentration of galactose as indicated by the absence of an increase in the peripheral blood galactose concentrations during a 2½ hour infusion Therefore this rate for the infusion of the galactose solution was selected for the comparison of the utilization of galactose by normal dogs and those

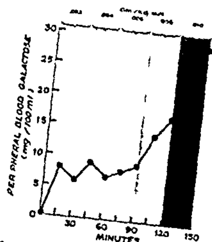


Fig 2 Graph illustrating the effect of increasing rates of administration of galactose into the portal vein of a normal dog

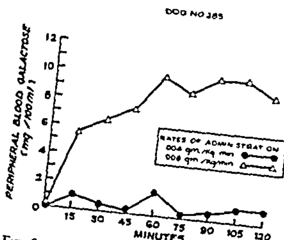


Fig 3 Graph comparing the relative effects of infusion of galactose in concentrations of 004 and 006 gm/kg/minute in a normal dog

with Eck fistulas or transposition of the portal vein and vena cava

In Figure 4 the results of infusions of the galactose solution at a rate of 004 gm/kg/min directly into the portal veins of normal dogs, dogs with Eck fistula and dogs with portacaval transposition are shown These data, which are the averages of the results in each group of animals studied in these experiments indicate that the utilization of galactose by the liver of dogs with portacaval transposition was almost exactly the same as that of the normal dogs but the utilization of galactose by the liver of dogs with Eck fistula was impaired The results suggest that hepatocellular function, as indicated by galactose clearance, is unchanged in dogs with portacaval transposition but is impaired in dogs with Eck fistulas

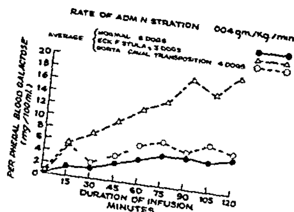


Fig 4 Graph showing comparison of clearances of galactose when infused at a rate of 004 gm/kg/minute directly into the portal vein of normal dogs dogs with Eck fistula and dogs with portacaval transpositions

SUMMARY

Plastic catheters were placed in the portal veins of normal dogs dogs with portacaval anastomosis (Eck fistula) and dogs with portacaval transposition in such a manner that galactose solution could be infused directly into their portal veins 6 or 8 weeks later without the use of anesthesia The rate of utilization of galactose by the liver of dogs with portacaval transpositions was similar to that of normal dogs, but it was decreased in animals with an Eck fistula

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EFFECT OF DIETARY CHOLINE ON BILIARY PHOSPHOLIPIDS EXCRETION *

FUMIO NAKAYAMA AND CHARLES G JOHNSTON

Reexploration of the common duct for residual stone is one of the most troublesome operations in abdominal surgery and is rather disappointing for patients For this reason much effort has been exerted in seeking satisfactory nonoperative treatment using ether chloroform and other drastic chemicals to dissolve stones In 1957 we found that phospholipids added to bile has a definite effect on the solution of gallstones *in vitro*[1] Therefore several attempts have been made to study the metabolism of biliary phospholipids[2,3] using radioactive phosphorus as a tracer in support of our clinical studies

Choline is an essential part of biliary phospholipids which have been believed to be composed of lecithin[4,5] It has been well known that choline accelerates phospholipid turnover in the liver[6] and is also incorporated into liver phospholipids[7] Therefore the present study was carried out on the metabolic relationship between dietary choline and biliary phospholipids

METHODS

Three μc of choline chloride (methyl C^{14}) (specific activity 2 03 mc /mM lot No 3 Nuclear Chicago Corporation Chicago Illinois) was administered to albino rats through a common duct cannula[3] in the distal end of the severed duct Bile samples were collected at one hour intervals from the proximal duct Phospholipids were extracted from the bile with chloroform methanol mixture (2 1) followed by the extraction with distilled water to remove inorganic phosphate free choline and bile salts as described in a

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* From the Department of Surgery Wayne State University College of Medicine and the Detroit Receiving Hospital Detroit Michigan Supported in part by a grant from the National Institutes of Health A 659 (c3) and A 699 (c3) Parke Davis and Co and the Research Corporation of Detroit Receiving Hospital

previous communication.[3] Total choline was precipitated as reineckate by the method of Glick.[8] Phosphorus was determined according to the method of King.[9] Radioactivity measurements were made in the usual manner with the use of a proportional counter (model PC-1, Nuclear Measurement Corporation, Indianapolis, Indiana).

To study the incorporation of dietary choline into the individual biliary phospholipids, 2 rats were given large doses of radioactive choline (in total 20 μc) and the bile was collected for an eight hour period. The biliary phospholipids extract obtained as above was subjected to chromatography according to Borgström [10] to remove neutral fat, fatty acid, sterol and some part of the bile pigments. Subsequently, the methanol eluate which contains phospholipids was concentrated under diminished pressure in an atmosphere of nitrogen and was subjected to the silicic acid column chromatography according to Hanahan, Dittmer and Warashina [11] *using 30 grams of silicic acid. The size of the fractions collected was 8 ml.*

RESULTS

After the administration of radioactive choline (methyl-C^{14}) into the duodenum radioactivity began to appear in the bile within one hour, reached its maximum in the second to the third hour and decreased rather rapidly. Over 94% of the radioactive carbon in the bile was recovered as choline. Choline which is incorporated in phospholipid will be found in the chloroform layer and free choline in the water layer of the bile extract.[3] Phosphorus in the chloroform layer represents phospholipid phosphorus in the bile. Therefore, the ratio between radioactive carbon and the phosphorus in the chloroform layer will be a direct measure of the incorporation of dietary choline into the biliary phospholipids, especially since 100% of radioactive carbon is recovered as choline reineckate. As shown in Figure 1, the incorporation proceeded quite rapidly, reached its maximum from the second to the sixth hour and decreased gradually. About 1% of the total radioactive carbon administered as choline (methyl-C^{14}) was recovered in the bile within 10 hours. Radioactivity was found in the water layer of the bile extract only in the first 3 hours and was a very small amount which accounted for about 5% of the total radioactivity excreted in the bile.

For study of the incorporation of dietary choline into the individual biliary phospholipids, each fraction of biliary phospholipids from the silicic acid column was subjected to phosphorus and radioactivity determination. Figure 2 shows the distribution of biliary phospholipids in the silicic acid column chromatography eluate. The main peak was identified as lecithin from the ratios of phosphorus, nitrogen, fatty acid and choline. Figure 2 clearly demonstrates incorporation of dietary choline into biliary lecithin *in vivo*, since the distribution of radioactive carbon, which was proven to be mostly in the choline molecule, was mainly found in the lecithin peak and both of the curves are quite similar (choline and phosphorus ratio in the lecithin molecule is 1·1).

DISCUSSION

Recently a considerable amount of evidence has been accumulated showing the incorporation of choline into lecithin in the presence of liver enzyemes by way of phosphorylcholine in which the presence of CTP (cytidine-5'-triphosphate) is necessary.[12] In the present experiment, after the

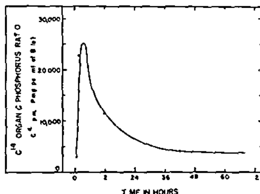


Fig 1 3 μc of choline (Methyl C14) was installed into the duodenum of rats and excretion of C14 and phosphorus in bile was followed The ratio between C14 and the organic phosphorus in chloroform layer of bile extract will be a direct measure of the incorporation of dietary choline into biliary phospholipids since 100% of the C14 in chloroform layer is recovered as choline reineckate Biliary phospholipids are mostly composed of lecithin in which the ratio between choline and phosphorus is 1 1

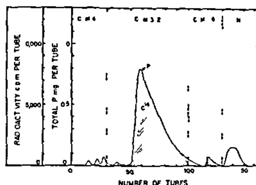


Fig 2 Chromatogram of biliary phospholipids from rats given in total of 20 μc of choline methyl C14 intraduodenally on 30 gm silicic acid 15 gm Hyflo Super Cel column ________ shows total phosphorus _ _ shows C14 The eluting solvents were mixtures of chloroform and methanol (v/v) C14 which is proved to be mostly in the form of choline was found mainly in the main peak which was identified as lecithin

administration of radioactive choline (C14 methyl labeled) into the duodenum liver phospholipids are chromatographed on the silicic acid column according to the method of Hanahan Dittmer and Warashina [11] Radioactivity over 98% of which is found in the choline molecule is detected in the lecithin and the sphingomyelin peaks and not in the phosphatidyl ethanolamine peak nor in the phosphoinositide peak This result indicates the incorporation of dietary choline into liver lecithin and sphingomyelin The incorporation of dietary choline into the biliary lecithin is also demonstrated (Fig 2) and this incorporation proceeds quite rapidly (Fig 1) Biliary phospholipids have been pointed out as an important factor in holding cholesterol in solution in bile beside bile salts [1] The present study reveals that dietary choline is absorbed rapidly from the intestine incorporated into phospholipids and excreted in the bile

SUMMARY

1 Radioactive choline (methyl C14) was administered into the duodenum of rats with common duct cannulae Radioactive choline is rapidly incorporated into liver lecithin sphingomyelin and biliary lecithin

2 Silicic acid column chromatography reveals that biliary phospholipids are mostly composed of lecithin

3 Phospholipid is an important ingredient of bile, and may play a part in stone formation

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FURTHER EXPERIMENTAL OBSERVATIONS ON THE ROLE OF STASIS OF THE EXTRAHEPATIC BILIARY TRACT IN THE GENESIS OF GALLSTONES*

Kamil Imamoglu, John F Perry, Jr, Harlan D Root,
Norman W Crisp, Conrad B Jenson, and
Owen H Wangensteen

Partial or complete obstruction of the cystic duct has been observed frequently in patients with cholelithiasis[5, 6] and anomalies and narrowing of the cystic duct have been reported to occur in as high as 65% of humans[8, 12] Cole and associates[5, 6] have suggested that partial obstruction of the cystic duct may be responsible for the occurrence of cholecystitis and by obstruction of the cystic duct have experimentally produced lesions in the gallbladder which resembled chronic cholecystitis of human patients Stones were found in one and possibly a second animal of their series

Experiments to be reported here were designed to determine if partial cystic duct obstruction alone or partial obstruction of the cystic duct combined with common bile duct narrowing resulted in gallstones in experimental animals

METHOD

The technique for experimental production of biliary stones has been described in detail[10, 11] Briefly, it consists in placing cellophane with dicetyl sodium phosphate around that portion of the extrahepatic ducts to be obstructed The resultant fibrosis causes partial obstruction of the duct in the majority of animals so treated Dogs and rabbits have been utilized in the ex

tion of both cystic duct and distal common bile duct was produced The desired result in both preparations was narrowing but not complete obstruction of the ducts in order to simulate as closely as possible the anatomical situation reported to occur in some humans with gallstones No special postoperative care has been given and animals have been fed regular laboratory rations

* From the Department of Surgery University of Minnesota Medical School Minneapolis Supported by USPHS Grant RG-4780 and anonymous donors

following surgery Spontaneous biliary concretions have not been observed in several hundred dogs and rabbits utilized for other experiments and fed this laboratory diet over long periods

All animals dying during the period of observation have been examined at autopsy and the others have been sacrificed at intervals At the time of postmortem examination the degree of narrowing of the ducts at the site of placement of the cellophane is determined In addition the gallbladder extrahepatic bile ducts and their contents have been examined In all animals sacrificed bacterial cultures from the gallbladder bile gallbladder wall and common bile duct wall have been carried out under aerobic and anaerobic conditions All stones were analyzed to determine their composition

RESULTS

Table 1 shows the results obtained with experimental cystic duct obstruction Although not the desired result complete obstruction of the cystic duct developed as a result of the stenosing agent in 2 of 11 dogs and 4 of 13 rabbits Calcium carbonate stones were present in the gallbladder of 4 of these animals with complete obstruction The remaining 2 rabbits with complete obstruction did not develop stones and the gallbladder was contracted and very small in each Nine rabbits with partial obstruction of the cystic duct all developed pigment cholesterol stones in the gallbladder Four of 9 dogs with partial obstruction of the cystic duct also developed stones of this same composition An animal was considered to have complete duct obstruction when bile could not be forced through the cystic duct from the gallbladder with manual pressure and a 1 mm probe could not be passed through the duct Animals with lesser degrees of cystic duct narrowing were placed in the group with incomplete obstruction

Routine histologic studies of the liver and common bile duct did not show any abnormalities in these animals In 2 dogs and 1 rabbit with incomplete cystic duct obstruction there was thickening of the gallbladder wall discernible grossly and chronic inflammatory changes were demonstrable by histologic examination In all animals with complete obstruction of the cystic duct the gallbladder wall was thickened and fibrotic in appearance

Five rabbits with partial obstruction of both the papilla of Vater and cystic duct developed stones in the gallbladder *(Table 2 page 524)* In 4 of these 5 rabbits stones were also present in the common bile duct Incomplete obstruction of distal common bile duct as well as the cystic duct developed in 5 dogs Three of these 5 animals had stones in the gallbladder and 1 of these 3 dogs had developed stones in the common bile duct when autopsy was performed at 59 weeks In none of these animals did the application of the reactive cellophane to both cystic duct and common bile duct cause complete duct obstruction All stones produced were of pigment cholesterol composition

Bacteriologic studies of bile and tissues removed at sacrifice showed bacterial growth in only a few animals Among the animals with partial duct obstruction a coagulase negative staphylococcus was cultured from the gallbladders and common bile ducts of 3 rabbits and 1 dog In 2 rabbits and 2 dogs with complete obstruction of cystic duct cultures of gallbladder wall showed a coagulase negative staphylococcus alpha streptococcus and a Bacillus species The remaining cultures were sterile

Table 1 Partial or Complete Obstruction of Cystic Duct

SPECIES	SURVIVAL TIME IN WEEKS	DEGREE OF OBSTRUCTION	STONES	COMPOSITION OF STONES	RESULTS OF BACTERIOLOGIC CULTURES
RABBITS					
1	51	Complete	+	Ca CO_3	Coagulase negative staphylococcus gram + aerobic bacillus
2	19	Complete	+	Ca CO_3	Coagulase negative staphylococcus Alpha streptococcus gram + aerobic bacillus in broth only
3	19	Partial	+	Pigment Cholesterol	Sterile
4	19	Partial	+		Sterile
5	19	Partial	+		Coagulase negative staphylococcus in broth culture only
6	19	Complete	—	— —	Sterile
7	19	Complete	—	— —	Sterile
8	19	Partial	+	Pigment Cholesetrol	Coagulase negative staphylococcus in broth culture only
9	19	Partial	+		Sterile
10	19	Partial	+		Sterile
11	19	Partial	+		Sterile
12	19	Partial	+		Sterile
13	19	Partial	+		Coagulase negative staphylococcus
DOGS					
1	23	Complete	+	Ca CO_3	Coagulase negative staphylococcus and alpha streptococcus
2	15	Complete	+	Ca CO	Coagulase negative staphylococcus and alpha streptococcus
3	48	Partial	+	Pigment Cholesterol	Sterile
4	19	Partial	—	— —	Sterile
5	19	Partial	+	Pigment Cholesterol	Sterile
6	19	Partial	—	— —	Sterile
7	19	Partial	—	— —	Sterile
8	18	Partial	+	Pigment Cholesterol	Sterile
9	15	Partial	—	— —	Sterile
10	15	Partial	+	Pigment Cholesterol	Coagulase negative staphylococcus
11	15	Partial	—	— —	Sterile

Table 2 Partial Obstruction of Papilla of Vater and Cystic Duct

SPECIES	SURVIVAL TIME IN WEEKS	STONES CBD	STONES CB	COMPOSITION OF STONES	RESULTS OF BACTERIOLOGIC CULTURES
RABBITS					
1	13	+	+	Pigment Cholesterol	Sterile
2	5	–	+	Pigment Cholesterol	†
3	44	+	+	Pigment Cholesterol	Sterile
4	43	+	+	Pigment Cholesterol	†
5	56	+	+	Pigment Cholesterol	Sterile
DOGS					
1	52	–	+	Pigment Cholesterol	Sterile
2	7	–	+	Pigment Cholesterol	†
3	59	+	+	Pigment Cholesterol	†
4	8½	–	–	– –	Sterile
5	4½	–	–	– –	†

† Culture not obtained

DISCUSSION

The role of stasis in biliary stone formation has been amply considered and discussed[1 2 3 4 5 6 7 9 11] Our previous experiments[10 11] and the present ones show that partial obstruction to the outflow of bile will result in stone formation in the biliary tree when bile contains bacteria or is free of them

As the gallbladder is the most common site of gallstones in man and as various types of obstructive lesions of the cystic duct in man have been described, such as anomalies of the valves of Heister, kinking and anomalous arrangements of the cystic, hepatic, and gastroduodenal arteries and of the bile ducts,[8] we desired to know if interference with the free passage of bile through the cystic duct would result in stone formation in the gallbladder It is of interest that in 18 of 24 patients with gallstones examined at autopsy, it was possible to demonstrate some degree of obstruction of the extrahepatic biliary tree which may have impeded the outflow of bile and 10 of these had narrowing of the lumen of the cystic duct such that a 1 mm probe could not be passed Obviously, anatomical obstructing lesions kinking the cystic duct do not account for the occurrence of gallstones in all human cases However, the above experiments do indicate that stones form in the biliary tree as the result of stasis alone, and metabolic and bacterial factors need not be implicated in their genesis Further investigations are necessary to determine if stasis as a result of anatomical or functional abnormalities of the bile ducts is responsible for gallstones in human patients

SUMMARY

Experimental stenosis of the cystic duct and of the cystic duct and ampulla of Vater has been produced in dogs and rabbits The majority of animals so

treated developed stones above the level of obstruction and in the majority, cultures of tissues of the biliary tree and the bile were sterile The experiments indicate that stasis with or without bacterial intervention will result in gallstones

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STUDIES IN PANCREATIC EXOCRINE INSUFFICIENCY *

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This study was carried out to evaluate pure pancreatic exocrine insufficiency in the dog as measured by the hydrolysis and absorption of glyceroltrioleate labeled with I 131 As described below several methods were used to exclude pancreatic juice from the intestinal tract while maintaining normal carbohydrate metabolism This allowed the consideration of pancreatic exocrine insufficiency as an isolated factor free of the many complicating abnormalities so often found in human patients

METHOD

Exclusion of the external secretions of the pancreas from the intestinal tract was accomplished in 32 dogs by the following methods 1) total pancreatectomy was performed in 4 dogs Diabetes was controlled by the administration of 10 to 15 units of NPH insulin daily 2) Resection of 90% of the pancreas was accomplished in 2 dogs leaving the residual 10% centered around the main pancreatic duct without any attempt to control the resultant diabetes 3) Ninety per cent of the pancreas was separated from the duodenum of 3 dogs leaving 10% of the gland centered around the main pancreatic duct Complete separation of the major portion was guaranteed by wrapping both the pancreas and the duodenum in separate portions of the omentum At a later date the main pancreatic duct was ligated and divided 4) One hundred per cent of the pancreas was separated from the duodenum of 8 dogs Omentum was interposed between the separated pancreas and the duodenum to assure continued separation 5) Total

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diffuse obstruction of the ductal system was produced in 6 dogs by injecting 2 cc of vinyl plastic in the main pancreatic duct [2] 6) Partial ductal obstruction was produced in 8 dogs by injecting 1 to 1 5 cc of vinyl plastic

The following studies were done on each of the dogs (a) the level of radioactive glyceroltrioleate (Triolein) in the blood of the dogs was measured at 3, 4, and 5 hours after the ingestion of a test meal containing 0 1 cc Triolein labeled with 15 microcuries of I_{131} in a gelatin capsule containing 0 5 cc of peanut oil [1] These blood radioactivity levels were determined before and at varying intervals after the production of pancreatic insufficiency (b) Weight curves and blood glucose levels were determined periodically (c) Frequent exploratory operations were done to evaluate the degree of pancreatic *disease* produced (d) Autopsies were done on the majority of animals

RESULTS

Group 1 Total Pancreatectomy Total pancreatectomy consistently produced an immediate drop in the Triolein uptake of 4 dogs The initial determinations done 5 to 8 days after surgery revealed blood radioactivity levels in the 0 to 2% range (Fig 1) Within 3 weeks after operation all uptake studies approached 0% levels Thus these low levels were noted at a time when the dog s general condition was still satisfactory All animals showed a progressive weight loss despite adequate control of diabetes

Group 2 Ninety Per Cent Pancreatectomy In 2 dogs removal of all but 10% of the pancreas produced a severe diabetes which was left uncontrolled until death 5 to 6 weeks after the operation The slight depression of Triolein uptake which occurred is shown in Figure 2 This decrease in uptake could probably be explained on the basis of the general debility of the dogs which were in acidosis at the time of the determinations Ten per cent of the gland appeared to be adequate to maintain normal exocrine function even though the basic endocrine function was greatly disturbed

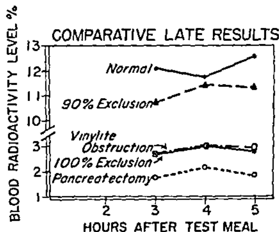


Fig 1 Comparative late followup results of Triolein absorption after various methods of excluding pancreatic juice from the intestinal tract

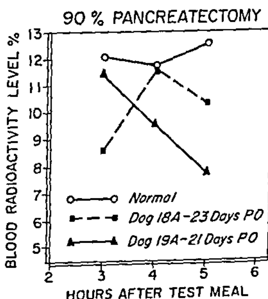


Fig 2 90% pancreatectomy did not alter the Triolein absorption levels

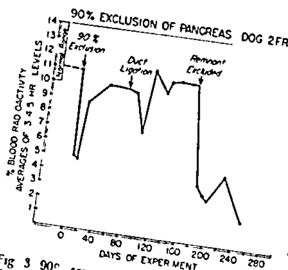


Fig 3 90% separation of the pancreas from the duodenum and subsequent ligation of the main duct of the pancreatic remnant produced transient decreases in Triolein absorption Following total exclusion of the 10% remnant there was a definite and lasting decrease

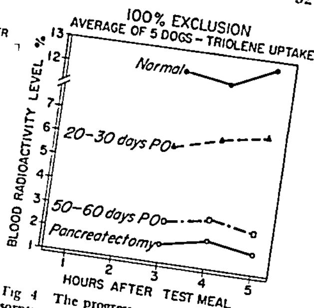


Fig 4 The progressive fall in Triolein absorption with time in dogs following complete separation of the pancreas from the duodenum

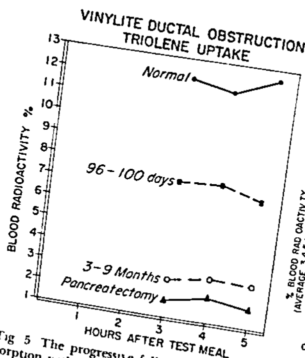


Fig 5 The progressive fall in Triolein absorption with time in dogs after diffuse obstruction of the pancreatic ducts with vinylite are shown

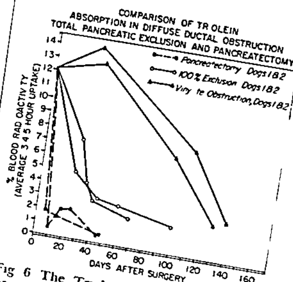


Fig 6 The Triolein absorption following pancreatectomy 100% exclusion of the pancreas and diffuse pancreatic ductal obstruction with vinylite are compared

Group 3 Ninety Per Cent Exclusion of the Pancreas On 3 dogs 90% of the pancreas was separated from its attachment to the duodenum It is noted in Figure 3 that the levels of blood radioactivity returned to normal after a transient fall often seen after any surgical procedure in the abdomen A high level of fat utilization was maintained even after the main pancreatic duct draining the 10% remnant of pancreas was ligated and divided Not until this remnant was completely separated from the duodenum did the Triolein levels fall The initial fall was immediate but a period of 30 to 40 days was required to reduce levels comparable to those seen after pancrea tectomy Weight curves in general paralleled alterations in Triolein levels All blood sugars were within normal limits

Group 4 Total Exclusion of the Pancreas When the entire pancreas was surgically separated from the duodenum there was an immediate fall in the absorption of Triolein This initial fall in 5 of 6 dogs was to levels of 5% to 8% (Fig 4) The sixth dog had lower levels but it had been necessary at the time of operation to ligate the principle blood supply to the pancreas With the passage of time a progressive fall in Triolein levels and body weight was noted in all 6 dogs until levels comparable to those in the total pancreatectomy group were achieved No evidence of diabetes was en countered during the period of followup

Group 5 Total Diffuse Ductal Obstruction with Vinyl Plastic The injec tion of 2 cc of vinyl plastic into the ductal system of the dogs pancreas pro duced a diffuse obstruction which appeared grossly and microscopically to be complete In a group of 6 dogs followed for 3 to 9 months a progressive fall in Triolein uptake was noted (Fig 5) An average of 30 days was required after obstruction for a fall in Triolein absorption to be noted Following this a gradual decline in fat utilization was observed and after 3 to 9 months the levels approached those seen following total pancreatectomy The rate of decline in Triolein levels was much slower than the animals in Group 4 (Fig 6) No abnormally elevated blood sugars were found

Group 6 Partial Ductal Obstruction with Vinyl Plastic In 8 dogs various volumes of vinyl plastic were injected into the pancreatic ductal system in attempts to produce localized obstruction Triolein uptake studies were quite variable but multiple exploratory operations revealed that in general the Triolein levels paralleled the degree of atrophy and fibrosis of the gland The striking finding was the maintenance of good levels of fat utilization when less than one tenth of the pancreas was left in a condition which might conceivably be producing pancreatic enzymes

DISCUSSION

It was noted early in this study that the pancreas possessed considerable reserve this was in agreement with observations of Coffey[3] and Dragstedt[4] The elimination of an estimated 90% of the pancreas from continuity with the intestinal tract was compatible with normal or near normal Triolein uptake as well as with the maintenance of body weights This 90% reserve of pancreatic tissue must be eliminated before any measurable degree of pancreatic exocrine insufficiency ensues This suggests as did the work of Coffey that human pancreatic deficiency states are seen only in very advanced destruction of that gland

The most interesting finding in this study was the delay in appearance of

pancreatic insufficiency under certain conditions After completely occluding the ductal system of the pancreas with vinyl plastic, a period averaging 30 days passed before the Triolein uptake levels began to fall. During this period the general condition of the animals was good and weight loss was minimal. A period of from 3 to 9 months was required before Triolein levels approached those of total pancreatectomy (Fig. 6). During this interval the progressive fall in fat uptake with coincident less of weight was parallel to the atrophy and fibrosis of the acinar tissue of the pancreas. Under the conditions of this experiment it might be possible that small amounts of pancreatic juice reached the intestinal tract around the plastic casts in the duct system That this explanation is only partly true is shown by the findings in the animals in which the entire pancreas was separated from the duodenum. In these 6 dogs there was an immediate drop in Triolein levels but the uptake was still considerably greater than those seen after pancreatectomy. Within 60 days a further reduction in Triolein uptake was noted with the levels approaching those of pancreatectomy.

Coffey in 1910 described a similar delay in the appearance of pancreatic insufficiency He credits the work of Lombroso [5] in 1901 as the first to demonstrate a difference in fat absorption between pancreatectomy and other means of total elimination of pancreatic secretions from the intestinal tract It was Lombroso who first proposed a pancreatic hormone which directly affected the utilization of foods One possible explanation offered by Coffey was the presence of small ducts between the pancreas and the duodenum which were left after his *ligation and avulsion* of the major ducts This possibility was eliminated in the exclusion experiments by the total separation of the pancreas from the duodenum That such channels do exist was well demonstrated in the 3 animals in which 90% exclusion of the pancreas was followed by main duct ligation with no significant decrease in Triolein absorption The only possible route for pancreatic enzymes was through small ducts between the duodenum and pancreas

Another possible explanation for the delayed appearance of marked pancreatic exocrine insufficiency would be the existence of some endocrine factor arising from the pancreas influencing fat utilization Dragstedt *et al* [4, 6] have published data supporting the thesis that a hormone *Lipocaic* is produced by the pancreas This hormone is effective in preventing the development of fatty livers in pancreatectomized dogs [6] Other investigators led by Chaikoff feel that the antifatty liver effect of pancreatic extracts is due to the presence of lipotropic substances in the extracts and that there is no real evidence that a specific hormone exists [7, 8, 9] The consistent finding in this study of the delay of marked pancreatic insufficiency would suggest the possible existence of a pancreatic hormone influencing fat utilization The development of a deficiency state in parallel to the appearance of atrophy and fibrosis within the gland might be construed to indicate that the *hormone producing cells* are gradually destroyed, thus producing a progressively more profound pancreatic exocrine deficiency.

CONCLUSIONS

1 Elimination of exocrine function but maintenance of the *endocrine* function does not produce pancreatic insufficiency of the same severity as rapidly as does pancreatectomy It is hypothesized that there might be an

endocrine factor affecting triglyceride uptake from the intestinal tract

2 Ten per cent of the pancreas appears to be sufficient to maintain normal exocrine function

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TRYPSIN INHIBITOR LEVELS IN EXPERIMENTAL PANCREATITIS *

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Under normal circumstances a part of the exocrine product of the pancreas can be found in the circulation indicating that there is a physiologic back diffusion of pancreatic secretions across the interstitial space Under experimental conditions when the amount of this back diffusion is large or when this is combined with an impaired blood supply either at the cellular or organ level pancreatitis will ensue

Trypsin is considered to be one of the responsible etiologic agents whereby pancreatic necrosis and hemorrhage occur The amount of active enzyme present will influence the degree of inflammation that can result There exist in the serum specific glycoproteins which are capable of binding trypsin and forming an inactive complex Under normal conditions it is presumed that trypsin inhibitors unite with the trypsin being diffused across the interstitial space But it is also thought they might represent a common agent to the apparent multiple etiologies of this disease so that the effective concentration of trypsin inhibitor available to the gland would have to be exceeded in order for the pathophysiologic process of pancreatitis to begin

METHOD

Mongrel dogs weighing between *14 and 20 kg were used Under nembutal* anesthesia they were sterilely prepared and draped and a right subcostal incision was made Ten cubic centimeters were aspirated from the gallbladder The puncture site was then closed with a single mattress suture of fine silk *The major pancreatic duct was then isolated* essentially without disturbing the gland itself The duct was ligated at the point of entry into the duo

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denum. The duct was cannulated with a #20 blunt nosed needle in a retrograde direction. Care was taken to remain distal to the immediate branches of the duct. The bile, in a 10 cc. syringe, was injected as quickly as the plunger could be depressed. The distal segment of the duct was then ligated and the abdomen closed.

In experiments where samples were taken from the venous drainage of the gland, the pancreaticoduodenal vein was isolated at its junction with the superior mesenteric. A small rubber shod clamp was used to temporarily occlude the vessel. Samples were taken from the lumen using a small gauge needle. Samples were also taken simultaneously from the inferior vena cava. In experiments where the abdomen was immediately closed following the injection of bile, samples were taken from the external jugular only. In each instance the development of pancreatitis was prompt and early hemorrhage within the interlobular septa could be seen. Control dogs were treated in an identical fashion except that after isolation of the duct, the abdomen was closed. In one group, samples were taken at 5, 20, 50, and 110 minutes after injection of bile. In the other group, samples were taken every 2 hours for 12 hours.

The specific details for the measurement of trypsin inhibitors are to be published at a later date. In general, the method is a modification of that reported by Jacobsen.[1] It involves the use of a hemoglobin substrate as reported by Anson[2] in which the amount of trypsin activity is measured in a spectrophotometer as a function of the absorbance of the split products that result from its incubation with the substrate. The stock trypsin solutions are then incubated with appropriate dilutions of serum and the difference in tryptic activity is a measure of the trypsin inhibited. The results of the inhibition are then standardized according to a factor derived from the inhibition of trypsin by a pure preparation of soy bean trypsin inhibitor.

The oral administration of trypsin has been demonstrated to be an effective means by which the serum trypsin levels could be raised in mice.[3] This method was adapted to dogs. Pure crystalline trypsin, 250 mg., (lyophilized and 2x crystallized) was fed to dogs twice daily in capsule form, for a perio of 2 weeks. The mean inhibitor level for 3 dogs so treated was raised 20% above the normal value of untreated dogs.

RESULTS AND DISCUSSION

The mean value for the serum trypsin inhibitor in 18 dogs was found to be equivalent to 522 ug of soy bean trypsin inhibitor/cc. The standard deviation is plus or minus 15.5% of the mean. Figure 1 represents the mean values of 4 control dogs. It will be seen that they fell within the normal range during the entire period of the experiment. All dogs in this group survived.

Experiment 1 was a short term preparation (samples taken over a 2 hour period only and from both the pancreatic and peripheral veins). Figure 2 represents the mean of five dogs in this group. There was no significant change in the peripheral levels during this period. However, blood draining from the gland demonstrates a significant fall in the concentration of available trypsin inhibitor.

In experiment 2, samples were taken every 2 hours for 12 hours and only from the peripheral circulation. It was not until 8 hours after the onset of

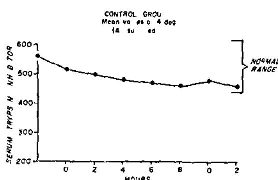


Fig 1 Control dogs

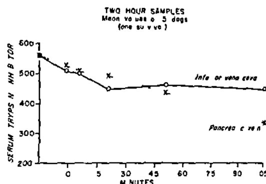


Fig 2 2 hour samples in 5 dogs

the disease that the peripheral trypsin inhibitor concentration fell significantly This then remained below normal levels during the remainder of the experiment Figure 3 is the mean of nine dogs in this group

In experiment 3, trypsin fed dogs were used There was no significant fall in the inhibitor levels during the course of the pancreatitis Two of the 3 dogs so treated survived the experiment as compared with 2 survivors of the 14 dogs that were untreated Figure 4 shows the mean values of the three dogs in this group

The oral administration of trypsin in addition to raising the trypsin inhibitor level may have other actions as well The addition of this proteolytic enzyme to the gastrointestinal tract may result in a readjustment in the amount of this enzyme that would be secreted by the gland Thus the effect of increased trypsin inhibitor levels may have to be considered in this light, and the final apparent protective effect of oral trypsin may represent a combination of these factors

CONCLUSION

1 Trypsin inhibitor levels will fall, in both the blood draining the pancreas (within 2 hours) and in the peripheral circulation (within 8 hours) following the onset of bile pancreatitis

2 Increasing trypsin inhibitor levels by the oral administration of pure trypsin may confer protection on animals in whom bile pancreatitis is induced

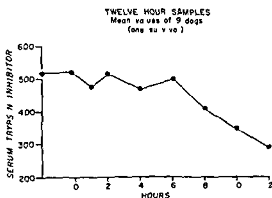


Fig 3 12 hour samples in 9 dogs

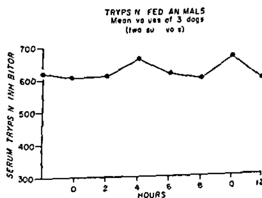


Fig 4 3 trypsin treated dogs—12 hour samples

3 The serum trypsin inhibitor may act as a defense mechanism so that the available concentration of this material around the gland must be exceeded by trypsin before the pathophysiologic process of pancreatitis can begin

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THE ROLE OF TRYPSIN AND OF BILE SALTS IN THE PATHOGENESIS OF ACUTE PANCREATITIS *

DAN W ELLIOTT, ROGER D WILLIAMS AND WILLIAM R C STEWART

Clinically it seems evident that disease in the biliary tree can precipitate acute and recurring pancreatitis but the mechanism by which this occurs has not been clearly understood According to the common channel theory of Opie occlusion at the sphincter of Oddi causes bile to regurgitate into the pancreas[1] Clinical and laboratory evidence has not fully supported this theory Recently, activation of trypsinogen within the pancreas by other means has been suggested as a cause of pancreatitis[2, 3]

Experiments previously reported from this laboratory implicate both bile and trypsin in the pathogenesis of this lesion[4] They have suggested a mechanism by which occlusion of a common channel may produce pancreatitis 1) pancreatic secretions enter and distend the biliary tree and 2) incubate with stagnant bile Pancreatic trypsinogen is activated and bile proteins are digested 3) Under any impulse the resulting mixture of bile and trypsin will reflux and infiltrate the pancreas at physiologic low pressures producing pancreatitis Normal bile without pancreatic trypsin will not do this Because of the current interest in trypsin alone as a cause of pancreatitis an effort has been made to evaluate the relative importance of bile and of trypsin when infused into the pancreatic ducts under physiologic conditions in producing this lesion

METHOD

In a series of adult mongrel dogs weighing between 11 and 15 kg the pancreatic ducts have been infused with solutions of trypsin of bile salts (sodium Taurocholate) and of the two combined An effort has been made to observe physiologic conditions in every respect during this infusion

Pressures within the pancreatic duct never exceeded 40 cm of water by direct measurement This limit was selected because it is well within the range of pressures observed in the biliary tree obstructed by spasm at the

* From the Department of Surgery The Ohio State University College of Medicine Columbus Supported by grants from the Comly Fund for Medical Research of the School of Medicine The Ohio State University and by a grant of the National Institute of Health

sphincter of Oddi[5] and only slightly above normal pancreatic secretory pressure which is about 30 cm of water in the dog

Bile salts were employed in the range of concentrations found in gall bladder and common duct bile 1% to 8% Sodium taurocholate was infused rather than whole bile in order that known concentrations could be employed Many years ago Flexner showed that bile salts are the most potent agents in normal bile for production of pancreatic necrosis[6] and this has been confirmed more recently[7]

The pH of each solution was adjusted to between 7 4 and 7 7 an optimum range for trypsin activity and frequently observed in normal bile

Commercial purified crystalline trypsin was placed in solution at approximately the concentrations found in dogs normal pancreatic secretions *Units of trypsin reported on commercial labels were found to vary widely* from the results of assay Therefore proteolytic activity was determined in each aliquot of solution employed and expressed in standard biochemical units[8] (1 unit of trypsin activity=the amount of trypsin which will release 04 mg of tyrosine from a 1% casein solution at a pH of 7 4 at 37°C in 10 minutes) All the trypsin concentrations and dosages reported in this paper are expressed in such units

Under aseptic conditions the major pancreatic duct was cannulated through the duodenum This was connected to a water manometer from which all solutions entered the pancreas To prevent loss of solution other pancreatic ducts were temporarily occluded by sutures all of which were removed at completion of the procedure The infusion was usually completed within 30 minutes

For comparison with the intrapancreatic administration of trypsin similar concentrations and dosages were also administered intravenously and intraperitoneally

All animals were autopsied Ordinarily if the procedure caused fatality it occurred in 24 to 48 hours After this period animals which appeared clinically well and resumed eating were counted as survivors and autopsied after 5 to 15 days Serum amylase determinations were obtained on all animals surviving 24 hours No antibiotics or other supportive measures of any type were employed

RESULTS

The results of trypsin administration alone are shown in Table 1 Generally under the physiologic conditions employed trypsin solutions will permeate the ducts and infiltrate the pancreatic parenchyma with great ease An edematous and hemorrhagic pancreatitis of some degree is produced with elevations of amylase

The severity of the pancreatic lesion and the mortality of this procedure appeared to be more directly related to the total quantity of trypsin administered in 30 minutes than to any other factor When for comparison similar concentrations and dosages of trypsin are administered in the same time interval by intravenous or intraperitoneal routes it appears that trypsin is no more toxic in the pancreas than it is systemically

When physiologic conditions are observed for the infusion of trypsin into the pancreas a significant lesion is produced only by massive and toxic quantities When less than 150 000 units were given minimal pancreatic lesions were obtained and only a slight mortality Nevertheless this quantity

of trypsin represents that found in at least 30 to 50 cc. of active pancreatic secretions. There is reasonable doubt that this much trypsin is present at any one time within the pancreatic parenchyma. Even if it were present, and activated by one of the mechanisms proposed,[2,3] apparently it alone would not cause significant pancreatic hemorrhage or necrosis.

The results of infusing bile salts in varying concentrations into the pancreas are reported in Table 2. Bile salts were placed in concentration at 8%, because this is about the maximum found in normal gallbladder bile. At this concentration, there is marked pancreatic resistance to infiltration under physiologic conditions just as there is to normal bile. As the concentration of bile salts is decreased, increasing quantities will infiltrate the pancreas. At concentrations found in common duct bile, 1 to 2%, large quantities will enter the pancreas but these produce little damage. Bile salts alone, under

Table 1. Tolerance of Dogs for Purified Crystalline Trypsin Comparison of Intrapancreatic, Intraperitoneal, and Intravenous Administration

ROUTE AND TOTAL DOSE GIVEN IN 30 MINUTES	NUMBER OF DOGS	NUMBER DIED	PER CENT MORTALITY
200,000 units or more			
† Pancreatic intraductal	4	3	75%
Intravenous	4	4	100%
150,000 to 199,000 units			
† Pancreatic intraductal	10	5	50%
Intravenous	10	6	60%
Intraperitoneal	6	3	50%
Less than 150,000 units			
† Pancreatic intraductal	12	1	8%
Intravenous	5	0	0

† Trypsin in saline at pH between 7.4 and 7.7, at concentrations between 3000 and 5000 units/cc., at a pressure never exceeding 40 cm. of water

Table 2. Permeability of Pancreas to Infiltration by Solutions of Bile Salts Infused in Ducts at Physiologic Pressure (40 cm. of Water)

PER CENT BILE SALTS IN SOLUTION †	CC. ENTERING PANCREAS IN 30 MINUTES AVERAGE	RANGE	NUMBER OF DOGS	PER CENT MORTALITY	ACUTE HEM. PANCREATITIS?
8%	5	(3 to 10)	4	0	Minimal
4%	10	(5 to 15)	8	12%	Moderate
2%	14	(7 to 20)	6	16%	Moderate
1%	28	(15 to 54)	6	0	Minimal

† Sodium taurocholate in saline at pH of 7.4 to 7.7

physiologic conditions apparently cannot be responsible for a significant pancreatic lesion

When trypsin at a relatively harmless concentration is added to bile salts in a medium concentration (4%) this solution will infiltrate the pancreas under standard conditions with increased ease A severe necrotizing and hemorrhagic pancreatitis results and is almost uniformly fatal Yet neither of these substances infused into the pancreas separately will produce a significant lesion These results are expressed in Table 3

Table 3 Additive Effects of Bile Salts † and Trypsin in Producing Acute Hemorrhagic Pancreatitis Infused into Pancreatic Ducts at Physiologic Pressures—40 cm of Water

	NUMBER OF DOGS	HEMORRHAGIC PANCREATITIS?	PER CENT MORTALITY
Trypsin † 75 000 to 150 000 units	12	Mod Severe	8%
Bile salts † 4% 20 cc	8	Moderate	25%
Bile salts † 4% plus Trypsin 75 000 units in 20 cc	16	Severe	93%

† Sodium taurocholate and/or crystalline trypsin 3000 to 5000 units/cc in saline at a pH of 7 4 to 7 7

There were moderate to extreme amylase elevations in all animals receiving bile salts combined with trypsin Death occurred in 24 to 48 hours and autopsy showed the typical findings of a fulminating acute hemorrhagic *pancreatitis with bloody ascitic fluid and extensive fat necrosis (Fig 1)*



Fig 1 Low power magnification of pancreatic section taken from dog 24 hours after intraductal infusion under physiologic conditions of a solution containing both trypsin and bile salts There is acinar necrosis interstitial edema and hemorrhage with early inflammatory infiltration

SUMMARY

These results appear to indicate that trypsin *alone* whether activated within the parenchyma of the pancreas or introduced into it by the pancreatic ducts under physiologic conditions will not of itself produce a significant hemorrhagic pancreatitis Tolerance of the dog for trypsin in the pancreas is as great as it is for trypsin administered intravenously or intraperitoneally

The pancreas resists infiltration by solutions of bile salts just as it does normal bile However when modest quantities of trypsin are added to bile

salts solutions there is increased permeability of the ductal system to the resulting mixture In the right concentration such a mixture of otherwise harmless bile salts and trypsin produces an extremely lethal pancreatitis The degree of pancreatic damage appears to be related directly to the nature and the concentration of the solution infiltrating the gland These observations afford additional experimental evidence in support of Opie s common channel theory for the pathogenesis of pancreatitis

A technique is described for the induction of an experimental acute hemorrhagic pancreatitis observing physiologic conditions with respect to concentrations of trypsin bile salts pH and intraductal pancreatic pressure This will produce a nicely standardized lesion that is almost uniformly fatal This technique should prove useful in the experimental study of therapy for this difficult disease

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CORTISONE IN THE TREATMENT OF EXPERIMENTAL ACUTE PANCREATITIS *

WILLIAM R C STEWART DANIEL W ELLIOTT AND
ROBERT M ZOLLINGER

Corticosteroid therapy has recently been reported to influence the course of acute pancreatitis favorably with sometimes dramatic improvement [1] However cortisone administered for the treatment of other diseases or experimentally apparently can be responsible for pancreatic inflammation and necrosis [2, 3, 4] Because of such conflicting reports an objective evaluation of the effect of cortisone on acute pancreatitis has been sought in the experimental surgical laboratory

A carefully standardized experimental acute pancreatitis has been induced in dogs by infusing a bile salt trypsin solution into their main pancreatic ducts utilizing principles previously described [5] and maintaining physiologic conditions throughout The lesion produced was treated in some animals with cortisone acetate and for comparison with concentrated salt poor albumin and with varying combinations of cortisone and albumin The

* From the Department of Surgery The Ohio State University College of Medicine Columbus. Supported by grants from the Comly Fund for Medical Research of the School of Medicine The Ohio State University and by a grant of the National Institute of Health

results were evaluated in terms of pathological changes in the pancreas quantity of bloody ascites produced survival rate and complications of treatment

METHOD

Adult mongrel dogs of both sexes weighing between 13 and 18 kg were used The procedure for inducing pancreatitis has been described in detail elsewhere[6] It consists of introducing into the main pancreatic duct under aseptic conditions a solution of trypsin and bile salts The pH concentrations and dosages of each are carefully regulated to coincide with those which might occur in a mixture of bile and pancreatic juice This solution is infused into the pancreas directly from a manometer at a pressure never *exceeding 40 cm of water This is well below maximum physiologic pressures* in the dog s biliary tree Under these conditions 20 cc of a solution containing 3100 to 3700 standard biochemical units of trypsin[7] and 4% sodium taurocholate will produce a lethal hemorrhagic pancreatitis

Treatment was begun in all but the control group as soon as the abdomen could be closed Cortisone acetate was administered intramuscularly to 25 dogs 100 mg immediately and 50 mg twice daily for 4 days after which it was gradually withdrawn

Concentrated salt poor serum albumin was administered to another group of animals to establish that the experimental pancreatitis produced would respond to appropriate treatment Previously it has been shown that sur vival of acute pancreatitis in dogs can be assured if quantities of albumin are given to prevent the acute blood volume deficits[8] known to occur with this disease In all dogs receiving albumin this was given intravenously at 3 sepa rate times immediately and at 8 and 16 hours after the induction of pan creatitis The dose at each administration was either 7 cc or 3 cc /kg of body weight In one group of animals albumin therapy at 3 cc /kg was combined with the standard cortisone administration

All animals succumbing to pancreatitis were autopsied as soon as possible after death Serum amylase was determined in those living more than 24 hours These were uniformly elevated to at least 3 times control values Once survival of the acute lesion seemed assured the animal was sacrificed and autopsied usually after 5 to 60 days

RESULTS

Table 1 summarizes the results in terms of survival in groups of untreated and treated animals High doses of albumin (7 cc /kg every 8 hours × 3) resulted in 100% survival while smaller quantities of albumin (3 cc /kg every 8 hours ×3) produced 50% survival *This appeared to indicate that the* standard acute pancreatitis induced was not so overwhelming as to defy vigor ous treatment

Cortisone acetate was administered intramuscularly to 25 animals as the only treatment given with a 48% survival rate Since untreated dogs almost uniformly die this improved survival rate suggests that cortisone either has some beneficial effect on the pancreatic lesion or provides some useful sys temic support under this stress The dosage employed is massive averaging 7 mg /kg of body weight There is little reason to believe that higher dosage would yield a better survival rate yet cortisone in maximum doses produces only one half the survivals attained with adequate albumin It was thought

Table 1 Treatment of Experimental Acute Pancreatitis

TREATMENT	NUMBER OF DOGS	SURVIVAL NO	%
None	16	1	6%
Albumin (7cc/kg × 3)	6	6	100%
Albumin (3cc/kg × 3)	10	5	50%
Cortisone (100 mg daily)	25	12	48%
Albumin (3cc/kg × 3) + Cortisone (100 mg daily)	10	4	40%

that if cortisone does provide useful systemic support it should reduce the amount of colloid necessary to achieve uniform survival

In a group of 10 dogs both cortisone at full dosage and albumin at half dosage (3 cc /kg × 3) were administered The 40% survival achieved is not different from that obtained with either agent used separately

Serum corticosteroid levels were determined in 7 untreated animals and showed a modest adrenal response to the stress of pancreatitis The levels at 12 and 24 hours were only 2½ times the preoperative values

In 5 animals treated with cortisone these levels averaged 10 times the preoperative value indicating good absorption in intramuscular cortisone deposits despite the vascular changes associated with pancreatitis

DISCUSSION

At autopsy of those animals succumbing in the first few days it was noted that after cortisone therapy the pancreas appeared grossly less indurated and less edematous than it did after no treatment or small doses of albumin

Bloody ascitic fluid was present in varying amounts in almost all dogs autopsied within one week following induction of pancreatitis This ascitic fluid was carefully measured by weight in the 15 untreated and the 13 cortisone treated dogs that failed to survive The untreated group averaged 345 cc of fluid with a range from 150 to 650 cc The average amount of ascites after cortisone was 138 cc and ranged from 0 to 200 cc Thus the untreated animals formed almost 3 times more ascitic fluid than those on cortisone therapy

Microscopic sections of the pancreas appeared to confirm the gross observation that cortisone therapy was associated with decreased edema and inflammatory reaction in the first few days This difference became much less apparent in animals autopsied at 5 or more days following induction of pancreatitis Nevertheless these observations suggesting that cortisone decreased the early inflammatory response in acute pancreatitis were for the most part made in animals succumbing to this disease

Two cortisone treated animals were sacrificed 1 and 2 months following recovery from pancreatitis In each animal the pancreas was markedly atrophic and appeared grossly as a thin cord less than 5 cm in diameter This appeared grossly and microscopically exactly like the pancreas in animals surviving similar periods after albumin treatment It appears that cortisone may produce a modest increase in survival rate by decreasing to some degree

the initial inflammatory response and ascitic exudation However *it does not* favorably alter the end result of the initial pancreatic damage

Of the 35 dogs receiving cortisone (or cortisone plus albumin) 8 were noted to have gastrointestinal hemorrhage and/or ulceration—an incidence of 23% This occurred only among the 21 dogs in this group surviving and receiving cortisone for at least 48 hours Among these the incidence of gastrointestinal complications approached 40% No ulcerations or hemorrhage were noted in the 32 dogs with acute pancreatitis that were not given cortisone

The most common lesions noted were multiple superficial punctate ulcerations of the stomach (but sparing the antrum) duodenum and jejunum A marked enteritis surrounded these lesions Seven of the 8 animals had ulcerations of this type and in 4 these lesions were responsible for significant gastrointestinal hemorrhage Bleeding was clearly responsible for death in two instances

Microscopically these lesions appeared to be superficial areas of mucosal necrosis not extending beyond the submucosa with moderate surrounding lymphocytic infiltration marked capillary congestion and hemorrhage (Fig 1)



Fig 1 Superficial gastric ulceration following cortisone therapy for experimental acute pancreatitis

One additional animal treated with cortisone and albumin survived acute pancreatitis but expired unexpectedly on the fifth day Postmortem examination disclosed a perforated gastric ulcer which measured 2 cm in diameter on the greater curvature just proximal to the antrum The recent origin of this ulcer was confirmed by pathologic sections

Altogether in the 35 animals receiving cortisone there were 19 deaths Three of these or one sixth were clearly attributable to ulcerative complications of steroid therapy Some of these ulcerations appeared after only 48 hours of cortisone administration at which time any gastric secretory response to steroid therapy was slight or absent Cortisone alone has not been reported to cause ulceration or hemorrhage in dogs[9] Acute experimental pancreatitis alone has not produced these lesions The combination of the two—cortisone and pancreatitis appears particularly likely to lead to dangerous gastrointestinal ulcerations

SUMMARY

A standardized fatal acute pancreatitis experimentally produced under physiologic conditions was treated with large doses of concentrated salt poor albumin Survival of 100% was achieved

Treatment of this lesion with cortisone in massive doses achieved 48% survival—possibly by modifying the initial inflammatory reaction In survivals

cortisone did not appear to alter the end result of the initial pancreatic damage

Cortisone does not achieve the good results of adequate early colloid replacement, nor does it reduce the amount of colloid necessary for survival

Cortisone given in the presence of experimental acute pancreatitis produced a high (38%) incidence of secondary gastrointestinal ulcerations and hemorrhage

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THE INTRACELLULAR ENZYMES RESPONSIBLE FOR PANCREATIC NECROSIS *

L. CORSAN REID, ROBERT E PAULETTE, AND J WILLIAM HINTON

Previously reported experimental work from this laboratory [1, 2] brought evidence that the mechanism of pancreatic necrosis in the experimental lesion or indeed the clinical disease, is the result of intracellular pancreatic enzymes acting upon any inadequately irrigated cells or tissues with which they come in contact The mechanism for the release of these degradative intracellular enzymes is the result of failure to deliver sufficient supplies to these cells to maintain cell membrane continuity and cell organization regardless of the multiplicity of factors which initiate these changes Work designed to recognize the enzyme responsible for these destructive changes in the hope of making possible some therapeutic control constitutes the basis of this report

The intracellular pancreatic disorganizing enzyme or enzyme system is associated with the microsomes and is entirely different from the enzymes in the pancreatic juice

METHOD

Pancreases were procured from dogs following a moderate bleeding until the organ blanched The pancreas was then placed in ice cold 0 25M sucrose solution and cut into small pieces These were homogenized in 0 25M sucrose in the Virtis The homogenate was always made in a 10% *concentration* The

* From the Department of Surgery New York University Postgraduate Medical School N Y C Supported by a grant from the John A Hartford Foundation

homogenate was then spun in a refrigerated centrifuge at 600 times gravity for 20 minutes The supernatant resulting from this spinning was spun at 7,500 times gravity for 20 minutes This supernatant was then spun at 20,000 times gravity for 1 hour This supernatant constituted the principal experimental material and was designated as the microsome fraction To isolate the microsomes, this microsome fraction was spun in the Spinco for 1 hour at 100,000 times gravity and the microsomes resuspended in sucrose and these, with the recovered supernatant were used as experimental agents

RESULTS

The microsomes on isolation had a highly destructive action on the oxidative energy release mechanisms in homogenates and isolated mitochondria as measured by the oxygen uptake and recorded by the usual manometric techniques The mitochondria were much more sensitive to this action than the whole homogenate Homogenates used were from myocardium and specific tissue from steer hearts, rat livers and kidneys, as well as spinach

The supernatant from the microsomes had a negligible effect on interfering with the oxygen uptake under similar conditions

The microsome fraction had a very destructive action on similar systems

When the microsome fraction was incubated in a suitable system with reduced hemoglobin, no splitting of the hemoglobin occurred Proteolytic activity was determined by digestion of denatured hemoglobin using the method of Anson [3] and measuring the trichloracetic acid soluble digestion products with the reaction described by Folin Ciocalteu [4]

When crystalline trypsin and hemoglobin are incubated together, splitting occurs

When the microsome fraction is incubated with trypsin, splitting occurs, as shown by the release of fragments which react with the color reagent

When trypsin, hemoglobin, and the microsome fraction are incubated, splitting occurs

When trypsin, hemoglobin, microsome fraction and crystalline trypsin inhibitor are combined, no splitting occurs

On addition of the microsome fraction to succinoxidase prepared from steer heart according to Clarke [6] in a system capable of demonstrating the reduction of cytochrome C in the presence of cyanate by the oxidation of succinate no effects could be demonstrated

A starch splitting enzyme was found in the microsome fraction

Desoxyribonuclease was present in the microsome fraction

DISCUSSION

The capacity of pancreas homogenates to disorganize their own energy mechanism and prevent oxygen uptake when measured by the usual manometric techniques can be transferred to homogenates of other organs and tissues such as myocardium and the specific tissue from steer hearts, rat livers or kidneys, and even spinach These activities are much more pronounced in the microsome fraction Mitochondria which is associated with the oxidative energy release mechanisms, are singularly sensitive to the action of the disorganizing factor In view of this finding, it was postulated that if the mitochondria were rich in phospholipids and the succinoxidase system were held together by the cement like action of the phospholipids, as suggested by

Dawson[5] then the most probable enzyme responsible for the disorganization would be a phospholipase Accordingly, succinoxidase was prepared from steer hearts according to Clarke This enzyme system showed good reduction of cytochrome C with succinate as measured at 550 mμ on the Beckman in the presence of cyanide However when the microsome fraction from the pancreas was added to this system no interference with succinoxidase activity occurred This would seem to exclude succinoxidase as the link in the oxidative chain which is affected by the degradative enzymes in the microsome fraction

To exclude trypsin as a factor in this activity, the experiments with reduced hemoglobin were done and the evidence is quite clear that this microsome fraction contains no trypsin or trypsin like enzyme There is present a starch splitting enzyme but this does not explain the disorganizing activity of the microsome fraction The finding of desoxyribonuclease in the fraction was a surprise and the present explanation would appear to be that in homogenization some nuclei were ruptured with release of desoxyribonuclease into the medium This does not satisfactorily explain the effects of the microsome fraction Our present position is that an esterase is involved and work is contemplated on its identification

SUMMARY

1 The microsome fraction of the pancreas does not contain trypsin or a trypsin like enzyme

2 The microsome fraction contains a starch splitting enzyme and desoxyribonuclease

3 The succinic oxidase system is unaffected by the microsome fraction

4 The microsomes on isolation contain the active enzyme or enzyme system responsible for the disorganization of the energy release mechanisms in homogenates or mitochondria from all other organs and tissues

We wish to express our thanks to Dr John Ayvazian for the determination of desoxyribonuclease

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THE ROLE OF THE PANCREAS IN IMMUNE BODY FORMATION *

ROBERT E PAULETTE, L CORSAN REID AND J WILLIAM HINTON

The pancreas is a most unique organ with a surprising number of important functions The extraordinarily wide range of these activities does not seem to be appreciated The idea that the pancreas might play a role in antibody formation evolved during an extensive study on the mechanism of necrosis in so called acute hemorrhagic necrotic pancreatitis [1] The results of an investigation of this possibility constitute the basis of this report

The pancreas plays a role, either directly or indirectly, in the synthesis of an important intermediate in the formation of antibodies

METHOD

Group 1 A comparison of antibody response to diphtheria toxoid in pancreatectomized dogs maintained on insulin to comparable controls was made

Group 2 Antibody response to diphtheria toxoid in pancreatectomized dogs maintained on insulin was compared to comparable controls, both groups receiving supplemental nitrogen in the form of oral amigen

Group 3 Antibody response to diphtheria toxoid in normal well fed animals was compared with animals starved for 5 weeks

Group 4 Homologous full thickness skin graft rejection following four separate transfers in pancreatectomized dogs maintained on insulin were compared with normal controls

The serum antitoxin titers were quantitated by the method of Fraser [2] using the rabbits skin neutralization test following comparable dilutions of toxoid by mixing known amounts of toxin with serum obtained from the experimental animals All control animals were maintained on the same replacement diet as the experimental group and those receiving amigen were given the same amounts per kilogram per day All dogs were short hair mongrels weighing 14 to 16 kg

Immunization Schedule. The minimum time of 4 weeks after pancreatectomy was established in all experimental animals before the introduction of the toxoid An alum precipitated diphtheria toxoid (1 ml) was given subcutaneously and a similar booster dose was given 3 weeks later At the end of 5 more weeks all animals were bled and serum obtained for antitoxin assay

The homologous full thickness skin grafts, each 3 cm in diameter, were transferred from back to back of donor to recipient and vice versa while identical autografts were done on the opposite side to act as controls

All dogs were autopsied and no pancreas was found in the pancreatectomized animals

RESULTS

Results are summarized in Table 1

The first group of dogs all maintained their weight and the controls produced twice the amount of antitoxin as the pancreatectomized animals In

* From the Department of Surgery New York University Postgraduate Medical School New York Supported by a grant from the John A Hartford Foundation

With the technical assistance of Mrs Olga Kekish

Table 1 Capacity of Pancreatectomized Dogs to Form Conventional Antibody

TYPE	NUMBER OF ANIMALS	TREATMENT OF ANIMALS	DIET	SERUM ANTITOXIN TITER IN U/ML
Test	3	Pancreatectomized	Replacement	5
Control	2	Normal	Replacement	10
Test	3	Pancreatectomized	Amigen + Repl	2.5
Control	3	Normal	Amigen + Repl	10
Test	5	Starved 5 weeks	Only Water	5–7
Control	2	Normal	Normal	5–7

the second group receiving amigen the controls produced 4 times the amount of antitoxin as the pancreatectomized animals In the third group the starved animals lost 31% of their body weight but there was no difference from the well fed controls in their capacity to produce antitoxin

The results of the skin graft experiments to determine the capacity to recognize and react against foreign tissues are summarized in Table 2

Table 2 Capacity of Pancreatectomized Dogs to Recognize and React Against Foreign Tissue

	NUMBER EXPER ANIMALS	TREATMENT	TIME OF HOMOGRAFT REJECTION—DAYS 1ST SET	2ND SET	3RD SET	4TH SET
Test	6	Pancreatectomy	Average 14 3 Range 13–15	Average 12.3 Range 11–15	Average 10.5 Range 9–14	Average 8.3 Range 9–11
Control	6	Normal	Average 11.2 Range 10–13	Average 8.2 Range 7–10	Average 6.3 Range 5–9	Average 5 0 Range 4–6

Ninety six full thickness 3 cm circular grafts were done half of which were homografts and half control autografts Twelve dogs were used 6 were normal and had been pancreatectomized for at least 4 weeks Each dog received 400 000 units of procaine penicillin G daily while the homografts were in place The set sequence of average rejection times in days for the pancreatectomized dogs was 14 3 12 3 10 5 and 8 3 while for the controls it was 11 2 8 2 6 3 and 5 0

DISCUSSION

These results suggest that the pancreatectomized animals are less efficient than the controls in the formation of antitoxin Starvation had no recognizable effe[illegible]

T[illegible]

g[illegible] of antitoxin as did the controls suggests there is a significant disturbance in protein metabolism The slower rejection time of

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DISCUSSION

These results suggest that the pancreatectomized animals are less efficient than the controls in the formation of antitoxin Starvation had no recognizable effect in altering the production of antitoxin as compared to the controls The failure of pancreatectomized animals to benefit from supplemental nitrogen in the formation of antitoxin as did the controls suggests there is a significant disturbance in protein metabolism The slower rejection time of

homologous skin grafts in the pancreatectomized animals as compared to the controls is very much in line with the findings of their capacity to form antitoxin.

Balch[3] in a clinical study, showed that only in those cases with severe lesions of the pancreas, such as carcinoma, was there failure of an adequate response to diphtheria toxoid In all other diseases studied there was a production of normal or better levels of antitoxin. This was true even in severely debilitated and patients desperately ill from different causes who had a better than average minimal response

One might suggest from this evidence that the pancreas plays a larger role in intermediary metabolism than is presently appreciated

SUMMARY AND CONCLUSIONS

1. Experimental evidence has been presented to support the hypothesis that the pancreas plays a role in antibody formation and the intermediary metabolism of proteins

2. Preliminary studies indicate there may be an associated impairment of the pancreatectomized dog's capacity to recognize and react against skin homografts as compared to controls

The authors express their appreciation to Dr A M Pappenheimer, Jr and Dr H S Lawrence for their advice and suggestions during the course of this investigation

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THE INFLUENCE OF ECK FISTULA UPON DIURESIS AND ANTIDIURESIS IN THE DOG *

ARTHUR J DONOVAN AND CHARLES G CHILD, III

Many early investigators of the physiology of the liver suggested that this organ plays an important role in regulating body fluid in normal animals and man For instance, in 1889 Johannson and Tigerstedt[1] wrote, "We see then a considerable quantity of fluid is taken up by the liver and thus withdrawn from the general circulation" A few years later Bayliss and Starling[2] showed that a considerable proportion of overloads of fluid was taken up by the portal circulation In patients with cirrhosis abnormalities in water distribution and excretion are of course widely recognized That water balance might be influenced by an Eck fistula has occurred to many students of hepatic physiology As recently as 1956 Eisenmenger and Nickel[3] reported that portal decompression by an end to side portacaval shunt exerted a favorable influence

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upon massive ascites occurring in patients with cirrhosis In our own experience we have studied 2 patients whose ascites, unaffected by other methods of treatment, subsided after an Eck fistula About 2 years ago we became interested in hepatic influences upon regulation of body water This report is concerned with experiments performed to determine whether an Eck fistula influences diuresis and antidiuresis in the dog

METHOD

Parenteral water loads were administered to normal dogs After determining their usual diuretic response and their subsequent antidiuretic response to surgical stress, exactly similar studies were performed upon dogs with Eck fistulae In performing these latter experiments we recognized that hepatic function is generally held to be impaired and splanchnic hemodynamics altered by an Eck fistula In order to differentiate between the influence of altered hepatic function and portal hemodynamics upon diuresis and antidiuresis, a further group of animals with Eck fistulae were studied in which the hepatic arterial blood supply was occluded just prior to giving the animals a load of water.

Normal Dogs. Water and food were withheld for 11 hours from 8 normal female dogs weighing between 10 and 13 kg They were then anesthetized with nembutal and given 100 ml of 5% glucose in distilled water per hour intravenously through a polyethylene catheter This had been inserted into an anticubital vein exposed by surgical dissection Bladder urine was collected every 30 minutes by gravity drainage from an indwelling catheter The volume and osmolarity of each of these samples were measured A fall in urine osmolarity below 200 mOsm/L with an associated rise in urine volume was accepted as evidence of diuresis An exploratory celiotomy of 30 minutes duration was then performed The subsequent antidiuretic response was documented by continued observation of urine volume and osmolarity All experiments were terminated when the peak of the antidiuretic response was passed The infusion of 100 ml of 5% glucose in distilled water per hour was continued until the end of the experiment At the initiation of representative experiments, during the diuretic phase, and during the antidiuretic response to surgical stress, blood was withdrawn for determination of serum osmolarity This blood was always obtained from a peripheral vein other than the one in which the polyethylene catheter had been inserted

Dogs with Preexisting Eck Fistula. Exactly similar experiments were performed in 12 dogs in which an Eck fistula had been established for at least 1 week

Dogs with Eck Fistula and Hepatic Artery Ligation. In 5 dogs a heavy silk ligature was passed about the hepatic pedicle when the Eck fistula was established In this a single loop was formed but not tied The free ends were brought through the anterior and posterior abdominal walls and buried in the subcutaneous tissue Just before the water load was administered these ends were retrieved and tied tightly With the exception of minor branches from the phrenic arteries or through the gastrohepatic ligament, the liver was thus deprived of afferent blood The remainder of these experiments was the same as those performed in normal and Eck fistula dogs The effectiveness of the hepatic arterial ligation was verified at the time of exploratory celiotomy or at postmortem examination

RESULTS

Normal Dogs. Immediately after anesthetization, catheterization, and surgical insertion of a catheter in an anticubital vein, 7 out of 8 normal dogs manifested a brief but prompt antidiuretic response This was reflected in rising urinary osmolarity Only 1 dog did not manifest this initial antidiuresis The maximum rise in urine osmolarity was within 1 hour in 6 of these normal dogs and at 2 hours in 1 dog (Fig 1) Diuresis as indicated by a fall in urinary osmolarity to 200 mOsm/L occurred in all 8 dogs within 3 hours of starting their water load (Fig 2)

Exploratory celiotomy always resulted in immediate water retention with an associated rise in urine osmolarity The peak of this antidiuretic response occurred about 2 hours after completion of the operation A fall in serum osmolarity from the level at the initiation of the water load was noted consistently in the course of the experiments

Dogs with Eck Fistula. Immediately after anesthetization, catheterization and surgical insertion into the venous cannula 9 out of 12 animals with Eck fistulae manifested an antidiuretic response This, however appeared later than in the normal animals The maximum rise in urine osmolarity was within 2 hours in 6 dogs, at 3 hours in 2 dogs, and at 3½ hours in 1 dog Figure 1 indicates time of maximum antidiuretic response to preliminary anesthetization, catheterization, and surgical insertion of a catheter in an antecubital vein in normal versus Eck fistula dogs

In 5 dogs with Eck fistulae diuresis did not occur and the experiment was terminated Diuresis occurred in all normal dogs In 7 dogs with Eck fistulae diuresis with a fall in urine osmolarity to 200 mOsm/L did occur at 3, 3½ 3½ 4 4, 4 and 5 hours respectively In all 8 normal dogs diuresis occurred within 3 hours (See Fig 2)

When a satisfactory diuresis did occur and an exploratory celiotomy was performed a prompt antidiuretic response developed This did not differ significantly from that observed in normal dogs

Dogs with Eck Fistulae and Hepatic Artery Ligation Immediately after anesthetization, catheterization, and surgical insertion of the venous cannula, 4 of 5 dogs with preexisting Eck fistulae and immediate ligation of hepatic arterial

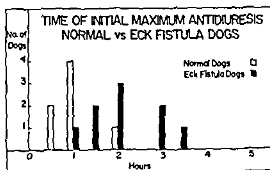


Fig 1 This bar graph depicts the time that maximum antidiuresis occurred following preliminary anesthetization catheterization and surgical insertion of a catheter in an antecubital vein in normal vs Eck fistula dogs

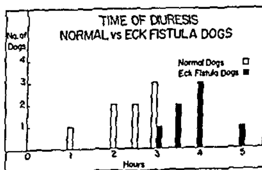


Fig 2 This bar graph depicts the time that diuresis was established in normal vs Eck fistula dogs

blood supply manifested an initial antidiuresis comparable to dogs with Eck fistula alone (cf Fig 1) In only one of these 5 dogs however was satisfactory diuresis subsequently established In 7 of the 12 dogs with Eck fistula alone a diuresis occurred The antidiuretic response to an exploratory celiotomy in the dog which did diurese did not differ significantly from that observed in normal dogs

DISCUSSION

Antidiuresis as one manifestation of the neuroendocrinological response to stress has been documented by Dudley *et al*,[4] by Hayes *et al*,[5] as well as by many others In our experiments the initial antidiuresis in normal dogs following anesthetization catheterization and surgical insertion of a catheter into the antecubital vein probably reflects the response of the dog to the stress of relatively minor procedures We as have others presume this to be a manifestation of increased secretion of antidiuretic hormone by the posterior lobe of the pituitary gland

Our most interesting observation concerns prolongation of this antidiuretic response to minor stress in dogs with Eck fistulae when these are compared to normal dogs It is clear from the data displayed in Figure 1 that the dog with an Eck fistula has a more prolonged antidiuresis following minor stress than does the normal dog This observation together with that indicating that diuresis in response to a water load (Fig 2) appeared later in the Eck fistula dog than in the normal animal has not been satisfactorily explained

That hepatic function in the dog with an Eck fistula is depressed has been suggested many times but its nature has never been clearly defined In our experiments we may suppose that the delay in diuresis and prolongation of antidiuresis in the Eck fistulae dogs is due to depression of liver function This concept is strengthened by our experiments in which the liver was functionally removed by both an Eck fistula and a ligation of the animal s hepatic artery At this time we can only suggest that as the function of the liver is compromised antidiuretic hormone exists in greater concentration than it does in the normal dog

Consider now the fact that we were unable to demonstrate any significant difference between the normal and Eck fistula dog as far as antidiuretic response to the stress of exploratory celiotomy was concerned This appears as a paradox and apparently contradicts our observation that the initial antidiuresis due to minor stress is prolonged and diuresis induced by water loads appears later in Eck fistula dogs than in normal animals In considering this several factors must be borne in mind In the normal dog antidiuretic hormone is believed to be secreted in response to three principal stimuli afferent neurogenic impulses such as occur following stress of even minor intensity increased serum osmolarity and decreased total body water Conversely, hypoosmolarity and increased total body water stimulate a diuresis presumably by inhibiting secretion of antidiuretic hormone Although neither is diuretic stimulus powerful enough to counteract the antidiuretic response that occurs secondary to afferent neurogenic impulses it is logical to assume that they might modify this response

What was the status of serum osmolarity and total body water at the time that the exploratory celiotomy was performed in these experiments? Analysis of our data indicates that serum osmolarity was depressed Specific data are not available with respect to volume of total body water However, diuresis

was established in all dogs by the administration of a constant infusion of 100 ml of 5% glucose in distilled water per hour Since diuresis was delayed in the Eck fistulae dogs and in dogs with Eck fistulae and hepatic artery ligation a greater increment in total body water occurred in these dogs than in normal dogs before exploratory celiotomy was performed Whether these factors of hypoosmolarity and hypervolemia modified the antidiuretic response to surgical stress in such a way that a pattern was obtained which did not differ significantly from that observed in normal dogs, has not been ascertained

SUMMARY

In dogs with Eck fistulae alone and particularly in animals with Eck fistulae and hepatic artery ligation, diuresis in response to a parenteral water load is delayed The antidiuretic response of these Eck fistulae dogs is accentuated when stress is inflicted before a diuresis is established Once diuresis is established in dogs with Eck fistulae, the antidiuretic response to exploratory celiotomy does not differ materially from that encountered in normal animals

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THE EFFECTS OF GRADUAL, COMPLETE OCCLUSION OF THE HEPATIC VEINS *

SAMUEL KLING AND PETER B R ALLEN

The basic physiology of portal hypertension and ascites remains obscure In 1951 Madden[1] working with corrosion specimens of cirrhotic livers postulated the primary factor in the formation of ascites is an obstruction of the outflow tract of the liver, namely, the hepatic veins In cirrhosis of the liver with irreversible ascites the obstruction is due to an obliterative fibrosis of the intrahepatic venous bed This concept was intriguing and stimulated speculation on what results could be produced by a gradual occlusion of the hepatic veins A survey of the literature disclosed little evidence of any work on the production of hepatic vein occlusion A technique which has finally been developed to occlude these veins is relatively simple, yet does consistently achieve the desired goal The purpose of this paper is to describe the technique in considerable detail and briefly summarize the effects produced by gradual, complete occlusion of the hepatic veins

* From the Department of Surgery and McEachern Laboratory University of Alberta Medical School Supported in part by a grant from the National Research Council of Canada With the assistance of Dr K Kowalewski and Dr Peter Davey

METHOD

Adult mongrel dogs weighing from 12.5 to 20.0 kg. were selected for this experiment. The animals were anesthetized with sodium pentobarbital (30 mg./kg. of body weight) intravenously and the anterior abdominal wall was prepared by the usual sterile methods. The abdomen was opened through a midline incision. The portal vein and inferior vena cava were identified and venous pressures in the vessels taken. To take these pressures a thin walled 15 gauge needle was introduced into the lumen of the vessel through an area of vein wall enclosed by a purse string suture of 4-0 vascular silk. The needle was connected by a short segment of rubber tubing to a simple water manometer both of which previously had been filled with a heparin saline solution. The base level of the manometer was judged to be at the level of the right atrium of the heart and all pressure readings were taken with this feature constant. Prior to withdrawing the needle from the portal vein 20 cc. of 70% diodrast (Iodopyracet Winthrop) was injected and a roentgenogram taken. The needle was then withdrawn and the purse string suture made taut so as to prevent an extravasation of blood from the puncture site.

A segment of the inferior vena cava cephalad to the renal veins was mobilized for a distance of 3 to 4 cm. Blalock vascular clamps were placed at the upper and lower ends of this segment of vein. A piece of thin walled wide bore polyethylene cannula was then selected. The piece chosen was of a length to reach from just caudad to the liver to a point just cephalad to the diaphragm (Fig. 1). A suture of 4-0 vascular silk was then tied on the caudal end of the cannula to subsequently act as an anchor suture for the cannula. The cephalad end of the cannula was tapered to facilitate its introduction into the inferior vena cava. Several small silver neurosurgical clips were placed on either end of the cannula to permit radiologic localization of the cannula when it was in place. The Blalock clamps were closed and an incision 1.5 cm. long was made in the anterior wall of this isolated segment of the vena cava. The bevelled end of the cannula was introduced into the phlebotomy incision while an assistant released the cephalad Blalock clamp so that the cannula was fully inserted into the lumen of the vessel. The anchor suture of the cannula with the needle still attached was brought out through the vein wall at the caudal end of the phlebotomy incision. This same suture was then used to close the incision in the wall of the vena cava. A simple running stitch was employed for this purpose. In earlier cases we had not anchored the cannula in this fashion and it was rapidly carried to the right atrium of the heart and

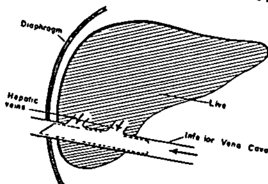


Fig. 1. Lateral projection of liver and inferior vena cava. Dotted lines illustrate position of plastic cannula. The wall of the cannula is in apposition to the ostia of the hepatic veins.

led to the death of the animals A neurosurgical type suction was found to be the best method for keeping the field dry during closure of the opening in the vena cava It should be mentioned that the polyethylene cannula selected was of the largest caliber which could conveniently be inserted into the vena cava The abdomen was closed in layers without drainage As experience increased we found the entire operative procedure could be carried out in approximately 40 minutes with negligible mortality

The following laboratory tests were done on each animal — (a) serum proteins with A/G ratio and electrophoretic fractionation, (b) serum sodium, potassium and chloride and (c) bromsulphthalein dye retention [6] These studies were done preoperatively and at 2 week intervals following the operation

When ascites became evident clinically the animals were reoperated upon Direct pressure readings were again taken from the inferior vena cava and portal vein In some cases the portal venogram was repeated When the animals were sacrificed necropsy specimens of liver, spleen, kidney and inferior vena cava in the region of the hepatic veins were taken

At necropsy, several dogs demonstrated a complete obstruction of the vena cava associated with the blockage of the hepatic veins The possible role of the vena caval obstruction in the production of the ascites and portal changes was, therefore, investigated and a control experiment devised Four dogs were selected and a short cannula of the same material was used It was placed in the vena cava just cephalad to the renal veins, but well caudad to the point of entry of the hepatic veins into the vena cava The same biochemical studies were made as in the main experiment

RESULTS

Four animals survived the operation but died within a few days before developing any evidence of ascites

Ten dogs developed clinically demonstrable ascites within 6 to 8 weeks of the operation In these the abdomen became markedly distended and tortuous venous collateral channels became prominent in the abdominal wall When the ascites reached full clinical proportions, the animals developed a pitting edema over the hind legs The amount of ascitic fluid present varied with the size of the animal, but ranged from 3500 to 5500 cc

The pressures in the inferior vena cava and in the portal vein taken at the time of the original operation in this group averaged 75 3 mm and 130 5 mm of water respectively When the animals were again operated upon after the establishment of ascites the pressures in these vessels averaged 90 5 mm and 260 mm of water respectively (Fig 2) It was interesting to note that in this group the portal systemic collateral venous channels were markedly dilated

At autopsy the portion of the vena cava which contained the plastic cannula was thickened and examination of its intima showed that the ostia of the hepatic veins had been obliterated by organized thrombi in the lumen of each vein Sections of the liver showed marked congestion in the central region of the liver lobules Fibrosis of the liver substance was not a prominent feature in any of our animals Sections of the spleen and kidneys revealed a moderate degree of congestion

The animals showed an over all decrease in total serum protein with a reversal of the albumin globulin ratio and an abnormal electrophoretic pattern

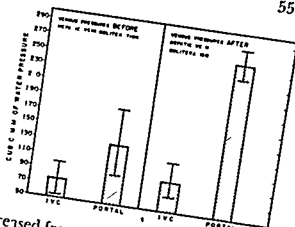


Fig 2 Pressures taken in the inferior vena cava and portal veins before and after obliteration of the hepatic veins

The bromsulphthalein dye retention increased from 10% to 70% as the ascites progressed Serum sodium, potassium and chloride showed little fluctuation and remained essentially normal throughout the procedure

The 4 control dogs remained healthy for a 6 month period following the operation There was no evidence of clinical ascites or portal hypertension and their biochemical tests remained normal When sacrificed the cannula was patent in 2 of the animals and in the other 2 it was completely thrombosed, indicating that in these 2 the factor of vena caval obstruction had been reproduced In none of these 4 animals was there any evidence of occlusion of the hepatic veins This would indicate that the pathology resulting in the original series was due to occlusion of the hepatic veins and not to occlusion of the inferior vena cava

DISCUSSION

Acquired data show that marked ascites and a degree of portal hypertension are produced in all animals in which this procedure is correctly employed It is necessary to stress the importance of placing the cannula accurately so that fibrosis of the ostia of all the hepatic veins is produced Failure to obliterate even one large hepatic vein may permit enough venous outflow from the liver to prevent the formation of ascites and portal hypertension We feel the control experiment described demonstrates that the important factor in the production of the ascites and portal hypertension is the occlusion of the hepatic outflow tract and that the inferior vena caval obstruction which occurs in some of the animals is not an important factor

This procedure is relatively simple technically, produces uniform results and provides a source of clinical and experimental material by which we may assess the relative merits of the types of therapy currently prescribed for these conditions

SUMMARY

A new method for producing gradual, complete occlusion of the hepatic veins is described Associated with this is the successful production of ascites and a degree of portal hypertension

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RESORPTION OF EXPERIMENTAL ASCITES IN THE DOG BY ILEOPERITONEOPEXY *

JOHN T WILSON AND CHARLES G CHILD, III

In 1955 Neuman Braunwald, and Hinton [1] reported that ascites produced in the dog by partially constricting the vena cava just above the diaphragm, could be controlled effectively by ileoperitoneopexy These investigators further showed that proteins absorbed from the peritoneal cavity by the exposed ileal mucosa were returned to the circulating blood volume and materially aided in maintaining serum albumin at normal levels About 2 years ago we became interested in how this type of ascites was transferred from the peritoneal cavity to the systemic circulation under the circumstances of this experiment We, therefore, repeated Neuman's original experiment and, after the ileal segment had become well established, detached it from its splanchnic blood supply and venous and lymphatic drainage The purpose of this report is to record the results of these experiments, for in our opinion they suggest the pathway by which this type of ascites is returned to the systemic circulation

METHOD

Constricting the thoracic vena cava was performed in 6 adult mongrel dogs In each animal the right thorax was entered through the seventh intercostal

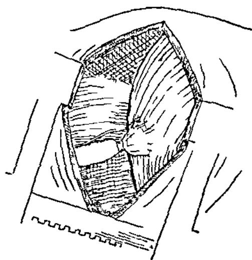


Fig 1 Artists conception of constriction of the thoracic vena cava with an aluminum band

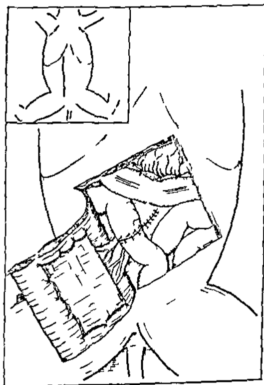


Fig 2 Artists conception of completed ileo peritoneopexy showing ileal segment open and sutured to the parietal peritoneum of the lateral abdominal wall

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space The cava was isolated just above the diaphragm and constricted to approximately one half of its normal diameter by an aluminum band 1 mm in width (Fig 1) Massive ascites developed in 1 to 2 months after banding the cava to this degree

Ileoperitoneopexy was performed in 4 dogs 1 month after the development of ascites and in 2 dogs 1 month prior to partial constriction of the cava One gram of sulfasuxidine was given to each animal orally for 3 days prior to fashioning their peritonealized ileal segments In each instance a 10 to 15 cm segment of terminal ileum was isolated and opened longitudinally along its antimesenteric border After washing the exposed mucosa with sterile saline solution the isolated segment of ileum was sutured to the parietal peritoneum of the right lateral abdominal wall with its *mucosal* surface facing the free peritoneal cavity (Fig 2) In all animals the greater omentum was amputated and intestinal continuity reestablished by end to end ileoileostomy At the conclusion of the ileoperitoneopexy 1 000 000 units of penicillin and 0 25 gm of streptomycin were instilled into the peritoneal cavity just prior to closing the abdominal wound

RESULTS

All animals survived both operations and were followed for at least 4 months after ileoperitoneopexy In 4 animals ileoperitoneopexy was performed after experimental ascites had become well established Ascites rapidly subsided in 3 The fourth animal of this group appeared so uncomfortable that 1000 ml of fluid were removed by paracentesis Rapidly thereafter its total collection of ascites subsided Of the 2 animals whose ileoperitoneopexies preceded constriction of the cava 1 never developed ascites while the other developed transitory ascites

The level of serum albumin appeared to be a sensitive index of function of the ileal segment The appearance of ascites was uniformly accompanied by a significant drop in serum albumin from an average of 3 1 gm /100 ml to 2 5 gm /100 ml or lower After successful resorption of the ascites by the ileal segments serum albumin levels rose to 2 9 gm /100 ml or higher One animal whose ileoperitoneopexy was performed prior to constriction of the cava *never* accumulated ascites and maintained a serum albumin level of 3 0 gm /100 ml or higher In Table 1 are tabulated the variations in serum albumin levels in

Table 1 Serum Albumin Levels in All Animals Before and After Operation

DOG	SERUM ALBUMIN GM /100ML.						
	BEFORE CAVAL CONSTRICTION	WEEK AFTER CAVAL CONSTRICTION			WEEK AFTER ILEOPERITONEOPEXY		
		1 wk	3 wks	6 wks	3 wks	6 wks	10 wks
1 †	3.8	3 0	3 4	3.3			
2 †	3 6	2 6	3 0	3 4			
3	3 0	2.3	2 7	2 7	2 7	2 8	2 9
4	3 4	3 0	2.5	2.2	2 7	2 8	3 0
5	3 2	2 4	2 4	2.3	2.5	2.5	2.9
6	2 9	2 7	2 6	2 6	2.9	3 1	3 0

† Ileoperitoneopexy performed before caval constriction

all animals before and after operations. Figure 3 graphically illustrates the protein changes in a representative animal from this series.

The degree of impedance of hepatic blood flow caused by caval constriction was estimated by splenic portography using cineflourographic techniques There was no evident change in portal flow after ileoperitoneopexy, despite complete resorption of ascites. The disappearance of ascites was not therefore due to decreased resistance to hepatic venous outflow.

The splanchnic blood supply and venous and lymphatic drainage of the isolated ileal segment was severed in three of the animals as early as 1 month and as late as 3 months after successful ileoperitoneopexy. Despite the fact that the segments were then parasitic on the systemic circulation of the abdominal wall ascites continued to be resorbed. In our opinion this indicates that ascitic fluid can be transferred from the peritoneal cavity to the circulating blood volume by way of the systemic circulation.

We studied the transfer of Evans Blue (4,4 Bis (7 (1-amino 8 hydroxy 2,4 disulfo) naphthylozol) 3,3 bitolyl) from the peritoneal cavity to the systemic circulation. In these experiments 20 cc of a 0 5% aqueous solution of dye diluted with saline solution to a total volume of 100 cc. were injected into the peritoneal cavity of normal dogs, of ascitic dogs, and of dogs having successful ileoperitoneopexies The thoracic duct was cannulated before injection of the dye and simultaneous determinations of dye content performed at 30 minute intervals on thoracic duct lymph and peripheral venous blood for a period of 3 hours. In normal and ascitic dogs the dye appeared in the thoracic duct lymph rapidly and in greater concentration than in the serum. In those animals with functioning ileoperitoneopexies established for from 2 to 3 months the dye appeared in the serum in concentrations comparable with those of the normal and ascitic dogs The concentration in the thoracic duct, however, was far less than in the normal or ascitic dogs. The decrease in thoracic duct lymph concentration in these instances may well be due to loss of the absorptive area of the greater omentum, since it was amputated during ileoperitoneopexy. Figure 4 graphically illustrates the average dye concentration curves for the various conditions of this experiment.

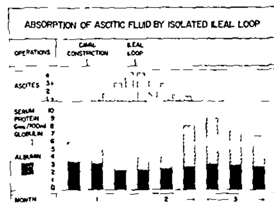


Fig. 3 Serum albumin changes before and after ileoperitoneopexy in a representative animal

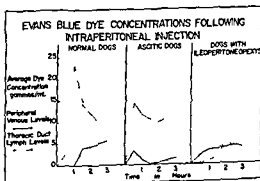


Fig 4 Average dye concentration curves for thoracic duct lymph and peripheral venous blood in normal and ascitic dogs and in dogs with functioning ileoperitoneopexies

SUMMARY

1. Experimentally produced ascites in the dog can be satisfactorily controlled by an isolated ileal segment sutured to the parietal peritoneum with or without its splanchnic blood supply intact.

2. Serum albumin levels seem to be a reliable index of function of the isolated ileal segments.

3. In our hands Evans Blue dye injection into the peritoneal cavity has not been useful in elaborating the mechanism by which experimental ascites is transferred from the peritoneal cavity to the systemic circulation, nor as a reliable measure of the rate of transfer.

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THE PRODUCTION OF EXPERIMENTAL ASCITES AND EXPERIENCES IN TREATMENT WITH THE RECTUS WICK OPERATION *

SIL H. LEE

In seeking a satisfactory method for the production of ascites in dogs with the express purpose of evaluating various surgical procedures reported to ameliorate the accumulation of intraperitoneal fluid, it at once became apparent that great variations in technique and uniformity of results exist in the literature. Occlusion of the thoracic segment of the inferior vena cava to varying degrees with a variety of materials would seem to be the most commonly used technique.

As early as 1915 Bolton[1] using pieces of Jacque's rubber catheter tied around the inferior vena cava, attempted to produce ascites in cats and monkeys. With complete occlusion of the inferior vena cava above the diaphragm, death occurred within a few hours. If, however, the occlusion was only about two fifths of the diameter, the constriction was not fatal and ascites occurred within 24 hours. Volweiler and his associates[2] reported the production of ascites within 3 weeks by the application of cellophane about the cava so that its lumen was initially constricted to one fourth of its original diameter. McKee *et al.*[3] using an aluminum band 1 cm. in width placed around the cava midway between the heart and the diaphragm so as to constrict the lumen of the vein to one half of its original diameter, produced ascites within a few days. Similarly, Laufman[4] applying an aluminum band at the level of the diaphragm so as to narrow the vein about 50%, produced a marked

* From the Dept. of Surgery, Laboratory of Experimental Surgery, University of Pittsburgh Medical School. Supported in part by U. S. P. H. Grant A 369 and the St. Francis General Hospital of Pittsburgh.

ascites which progressed for about 4 months and then gradually disappeared if the animal survived. Parson and Holman,[5] using umbilical tape to occlude the vein by 50% of its outside diameter produced ascites which had completely disappeared by 140 days. A 1/4" polyethylene band was used by Berman and Hull[6] to occlude the inferior vena cava to about one-half of its lumen. Ascites gradually occurred from the fourth to the seventh week postoperatively. Feeding a high salt diet (5 to 10 gm. daily) after partial caval occlusion with an aluminum band, Hyatt and colleagues[7] was able to produce massive ascites within 2 weeks after operation. Recently, liver cirrhosis with marked ascites has occurred following intravenously administered radioactive colloidal gold.[8] By this method dogs developed ascites about 6 weeks after injection The initial dose of intravenous radioactive colloidal gold was 10 to 13 mc., followed in 2 weeks by a dose of 19 to 20 mc., and 1 month later by a larger dose of 30 to 40 mc.

These and other investigators who successfully produced ascites in dogs or other animals, used materials and methods for determination of degree of occlusion of the vein which were markedly dissimilar. In many, the calculations of the percentage of partial constriction of the inferior vena cava in its thoracic segment are open to criticism. The size of the inferior vena cava varies greatly from animal to animal and, if partial constriction of that vein were to be made to the same caliber in all dogs, the degree of constriction and the length of time to produce ascites would not be comparable and the results would be different.

The purpose of this paper is to present a standardized technique for the production of ascites which will give predictable results. Further, experiences with the use of a modification of the "rectus wick" operation originally described by Crowe[9] for the control of ascites will be discussed.

METHOD

Production of Ascites. Twenty-eight mongrel dogs of both sexes, weighing 10 to 15 kg., were used. Animals were anesthetized with pentobarbital sodium and when the chest was opened, they were maintained on positive pressure respiration. Determination of the size of the vena cava and the degree of constriction to be made is illustrated in Figures 1-A and B. A large knot was placed about 4" from one end of a black silk suture. This was then passed around the vena cava so that the knot lay on the anterior surface of the vein. The other end of the ligature was approximated to the knot and, by measuring the length of the silk, the outside circumference of the vessel was obtained. Pieces of #50 polyethylene tubing were cut in lengths 1/3, 1/2, 2/3, and 1/4 of the vein's circumference, depending upon the degree of constriction to be created. Black silk, #3-0, was threaded through the polyethylene tube and tied around the vena cava so that the two ends of the tubing were approximated. Larger polyethylene tubing is undesirable as it kinks or folds. Further, tubing this size does not cut through the wall of the vein.

Since quantity of flow in a blood vessel is proportional to the fourth power of the radius, provided pressure and viscosity are constant, an idea may be obtained of the magnitude of change in cross sectional area of a vessel together with the quantity of blood passing through it by a specific reduction of the circumference of that vessel (Table 1).

Operation of Rectus Wick. Twelve ascitic animals were anesthetized with

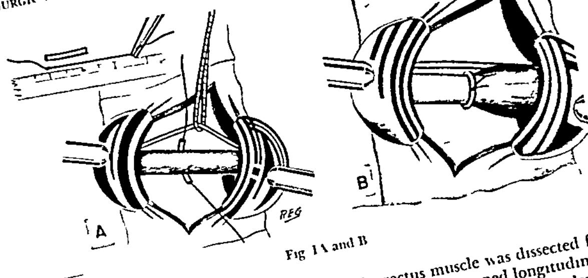


Fig 1A and B

open drop ether and either the left or right rectus muscle was dissected free from the rectus sheath and peritoneum The latter was opened longitudinally beneath the muscle and one border of the muscle was sutured to the underside of the peritoneum, following which, it together with the fascia were resutured over the muscle

In 4 animals with ascites the operation was performed as suggested by Crowe The right rectus muscle was dissected free of its sheath, severed at its costal margin and the cut end was attached to the posterior peritoneum just anterior to the sacrum

RESULTS

It was concluded (Table 2) that the most satisfactory method to produce ascites in dogs is to constrict the circumference of the thoracic inferior vena cava by one half This reduces the cross sectional area of the vein to one fourth of its original size All animals so treated developed marked ascites by 3 to 4 weeks after operation On the other hand if the circumference was reduced by less than 50% no ascites occurred in animals observed as long as 6 months, and decreasing it by more than one half resulted in the prompt death of all dogs

Five animals with ascites served as controls They were subjected to paracentesis approximately once a month at which time as much as 2,700 ml of ascitic fluid were removed Ascites promptly recurred following each "tapping" These animals survived 20 to 32 weeks Before death all exhibited a pic

Table 1 Relationship of Reduced Circumference of Inferior Vena Cava to Cross Sectional Area of This Vein

CIRCUMFERENCE REDUCED TO	CROSS SECTIONAL AREA (LUMEN) REDUCED TO	BLOOD FLOW REDUCED TO
2/3	4/9	1/5
1/2	1/4	1/16
1/3	1/9	1/81
1/4	1/16	1/256

Table 2 Effect of Partial Inferior Vena Caval Occlusion

NO DOGS	CIRCUMFERENCE REDUCED TO	RESULTS
2	1/4	Both dogs died within 4 hours
5	1/3	All dogs died within 6 hours
4	2/3	All observed up to 6 months No ascites
16	1/2	All developed marked ascites by 3 to 4 weeks

ture similar to that seen in portacaval shunt animals with meat intoxication It is important to emphasize that no spontaneous remission of ascites occurred in this group

Four animals with ascites of 3 months duration similar in degree to that found in the control group were subjected to a Rectus Wick operation as described by Crowe Following this operation ascites reaccumulated within 2 weeks One or two paracentesis were done following which all animals remained free of fluid for about 3 months There was then a gradual reaccumulation of ascitic fluid which was not ameliorated by further tapping over an additional 2 month period

These 4 animals and 8 others which were ascitic for 4 months and had been tapped similarly to those in the control group were subjected to the modified rectus wick operation as described above All 12 of these animals required one or two paracentesis after operation and then were seen to remain free of ascites for as long as 1 year when all were sacrificed

It is of interest to note that the rectus muscles in animals in which the Crowe wicking procedure was done were found to be atrophic and covered with peritoneum by the twelfth week after surgery In animals where the rectus muscle was not detached but transposed beneath the peritoneum no atrophy of muscle was seen and little envelopment of the muscle by the peritoneum was evident In some animals a few adhesions between the omentum and muscle were present Sections taken from livers of animals which survived one year after operation showed evidence of marked congestion but no cirrhosis

SUMMARY AND CONCLUSIONS

It has been estimated that between 50 and 80% of cirrhotics have ascites as a complication [10, 11] In many the latter can be controlled by rigid medical management In some however ascites is intractable and requires surgical intervention to provide symptomatic relief Because of dissatisfaction with simple paracentesis such procedures as ileal eversion hepatopexy and implantation of foreign material (Crosby Cooney button) have been attempted to alleviate the accumulation of fluid None has been generally acceptable The same may be said at least experimentally for the rectus wick operation as originally proposed by Crowe The severance of the rectus muscle and implantation of one end results in atrophy of the muscle However it would

seem that if the entire muscle is transplanted beneath the peritoneum maintaining its blood supply intact this procedure has merit

The most satisfactory way to produce permanent ascites in the dog is to reduce the circumference of the inferior vena cava by one half In all instances marked ascites will occur between 3 and 4 weeks

The author wishes to express his indebtedness to Dr Bernard Fisher for his continued encouragement and kind direction in this study

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AN ATTEMPT TO DETERMINE THE SOURCE OF EXPERIMENTAL ASCITIC FLUID *

Irving F Enquist Roy G Aiello, Bernard S Levowitz and Etsutaro Ikezono

It has been demonstrated that partial constriction of the inferior vena cava above the liver gives rise to the production of a profuse and sustained ascites Many investigators feel that this ascites represents lymph which has been extruded from the surface of the liver and that the ascites found in patients with severe liver disease also represents lymph from the liver Whether or not all of the ascitic fluid found in the experimental situation is hepatic in origin is not known The work of Gage and his collaborators [1] which showed that when the liver is encased in dense adhesions and the inferior vena cava constricted above the liver no ascites will form presents strong evidence in favor of the exclusive hepatic origin of this fluid Mallet guy [2] in order to present a critical experiment on the role of the liver in the production of this fluid attempted in dogs to move the liver into the chest and then to constrict the inferior vena cava He was unsuccessful in these attempts but was able to move one or more lobes of the liver into the chest and then to constrict their respective hepatic venous branches From the results of several preparations of this

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type, he and his group concluded the liver is the sole source of experimental ascitic fluid produced by vena cava constriction Although he presents no objective data Freeman[3] also has indicated that he and Ivy moved the liver *into the chest of dogs When the vena cava was subsequently constricted the* animals formed hydrothorax but no ascites It is our purpose to present objective evidence that in dogs in which the liver has been placed in the chest and the thoracic inferior vena cava constricted, most of the fluid accumulation occurs in the thoracic cavity

METHOD

Mongrel dogs, weighing 10 to 16 kg, were used Many earlier attempts were unsuccessful for various reasons, and after a lengthy trial and error period the following technique was found to be successful in approximately two thirds of the attempts On the day before operation the dog was given one million units of crystalline penicillin intramuscularly The operation was carried out under light ether anesthesia administered by a skilled anesthetist A long right thoracoabdominal incision was made, dividing the seventh and eighth ribs close to the sternum The diaphragm was split down to the inferior vena cava and the right phrenic nerve was divided, the incision was continued as an inverted Y,' each dorsal limb being carried down to the posterior wall The incision was carried along the posterior wall out to the mid point of the lateral wall bilaterally The left phrenic nerve was carefully preserved All hepatic ligaments were then divided but the structures in the porta hepatis were preserved In the first successful experiments, the diaphragm was then reconstituted after allowing the liver to fall into the thoracic cavity Although a water tight closure was not effected about the inferior vena cava and the structures in the porta hepatis, it was closed as tightly as possible After penicillin had been placed in the peritoneal and pleural cavities, the incision was closed leaving two water seal drainage tubes in the pleural cavities, these were removed after the animal had been allowed to recover from the anesthetic *(usually within 10 minutes) At least 400 cc of whole blood and two million* units of aqueous penicillin were given *intravenously to each animal during* the operation Postoperatively the dogs were allowed to eat and drink at will They were given intramuscular penicillin daily and were given infusions of dextrose solution and electrolytes as indicated Two to 3 weeks after the first operation the dogs were re operated upon through a left thoracotomy, and the inferior vena cava constricted to one half of its original diameter

Because of the high mortality associated with the second operation in animals with livers displaced into the chest, the procedure was modified in order to carry out the entire operation in one stage Therefore, in all subsequent animals a strip of reactive polyethylene was sutured snugly about the inferior vena cava during the original operative procedure This resulted in a gradual but definite constriction of the vena cava and obviated a second operation All animals were then observed until death or they were sacrificed 1 to 6 weeks after the operation in which the vena cava was constricted

RESULTS

Three animals survived a successful transplantation of the liver into the chest with subsequent partial ligation of the inferior vena cava Two of these animals died several weeks after the second operative procedure and the other was sacrificed The results of the autopsy examinations on these animals are

indicated in Table 1. It is important to point out that in none of these animals (and in none of 4 other animals who were subjected to liver transplantation and who died during the vena cava ligation) was an abnormal amount of fluid found in the thoracic cavity before the vena cava was constricted.

Table 1. Fluid Collections in Dogs Prepared by Two Stage Liver Translocation and Caval Constriction

ANIMAL NUMBER	VOLUME OF HYDROTHORAX (CC)	VOLUME OF ASCITES (CC)
471	500	30
1056	800	0
1120	650	100

By the present technique a strip of reactive polyethylene is sutured snugly about the vena cava at the time of the liver translocation. Eleven animals survived such an operation long enough (2 weeks or longer) to provide significant information, and the results of the autopsy examinations in these animals are shown in Table 2. In animals 211, 238, 283, and 1216 the diaphragm was intact at autopsy, the vena cava was constricted, and the thoracic cavity contained much more fluid than did the abdominal cavity. Animals 246, 253, and 346 presented equal volumes of fluid in the pleural and peritoneal cavities at autopsy. (Here, and in all subsequent discussion, the pleural cavity figure represents the total volume of fluid in the two pleural cavities. The mediastinum was broken during the operative procedure, but had become intact again and was water tight by the time of autopsy in all animals.) In one of these (253) there was a fistula between the left pleural

Table 2. Fluid Collections in Dogs Prepared by One Stage Liver Translocation and Caval Constriction

ANIMAL NUMBER	VOLUME OF HYDROTHORAX (CC)	VOLUME OF ASCITES (CC)
283	550	60
211	720	250
1216	700	0
238	900	160
246	350	350
253 †	700	780
346	420	400
214 †	50	0
1081	0	0
7	0	0
220	300	1100

† Pleuro peritoneal communication

and peritoneal cavities because of a break in the suturing of the diaphragm This defect appeared to allow free flow of fluid from one space to another. In dogs 246 and 346, however, the diaphragm was a complete, water-tight barrier.

There was essentially no fluid in any of the serous cavities at the time of autopsy in animals 7, 214, and 1081. In each of these animals the inferior vena cava was constricted beneath the polyethylene wrapping. In one (214) there was a break in the diaphragmatic suture, leaving a fistula between the left pleural space and the peritoneal cavity, very similar to that seen in animal 253, described above. The repair was intact in dogs 7 and 1081.

In dog 220 there was much more fluid in the abdomen than in the chest. In this animal the diaphragmatic repair had broken down over a small area dorsally (4 $\times$ 5 cm.) leaving a small lobe of liver exposed to the peritoneal cavity in the region of the lesser sac. This sac was distended with fluid, and the entire peritoneal cavity contained a total of 1100 cc. Because of dense adhesion formation within the chest, the involved lobe did not communicate with either of the pleural cavities.

DISCUSSION

With the exceptions noted, it was found that in animals studied at autopsy there was usually more fluid in the chest than in the abdomen. The translocation of the liver, without caval constriction, did not lead to hydrothorax. In several animals there was no fluid at all within the thorax at autopsy; this proves that the sheet of reactive polyethylene did not give rise to any abnormal fluid production.

The absence of fluid in any serous cavity in animals 7, 214, and 1081 is probably the result of several factors. Firstly, though the vena cava was constricted, it may not have been constricted sufficiently. Secondly, the gradual constriction of the vena cava might have allowed collateralization to occur, so that severe hepatic congestion did not result. Thirdly, (and this we believe likely,) the multiple dense adhesions between the liver and other structures within the thorax might serve as the routes for the development of numerous new lymphatic channels, sufficient to carry away the increased amount of hepatic lymph. These new conduits would be comparable to the new lymphatic channels which Gage and his associates [1] found after hepatopexies effected by liver trauma and poudrage. It is important to point out that in none of the animals in this experiment was the total volume of fluid in the serous cavities as great as the volume of ascitic fluid seen regularly in animals with caval constriction and abdominal livers. It appears, therefore, that the great number of dense adhesions about the liver, probably containing new lymphatic channels, was capable of decreasing the fluid production in the serous cavities of all animals with a thoracic liver and vena caval constriction.

Animal 220 perhaps demonstrates more graphically than the other animals the importance of the liver in the formation of experimental ascitic fluid. Mallet-guy [2] had found that the transposition of a single or several hepatic lobes into the thoracic cavity, with constriction of the respective venous drainage, would result in a profuse exudation of fluid into the thoracic cavity. Animal 220 seems to represent the reverse of the Mallet-guy experiment, because the space about the involved lobe did not communicate

with either of the pleural cavities, but did communicate freely with the peritoneal cavity via the lesser peritoneal sac.

We are unable to explain why animals 216, 253, and 316 presented equal volumes of fluid in the pleural and peritoneal cavities. In dog 253 there was a definite communication between the pleural and peritoneal cavities, so that the source of the fluid cannot be stated. The diaphragm was complete in the other 2 dogs, therefore, we must assume that the peritoneal fluid was of extrahepatic origin (weeping from peritoneum, leak from ruptured, enlarged lymphatic channel from liver?). This problem warrants further investigation.

SUMMARY

In dogs, the entire liver was moved into the thoracic cavity and the diaphragm resutured beneath it. After constriction of the inferior vena cava above the liver, more fluid accumulated in the chest than in the abdomen These results suggest that the primary source of ascitic fluid in animals with vena caval constriction is lymph which is extruded through Glisson's capsule

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THE TOXICITY OF PERITONEAL FLUID RESULTING FROM STRANGULATION OF VARIOUS SEGMENTS OF THE GASTROINTESTINAL TRACT *

RICHARD DOYLE AND WILLIAM O BARNETT

The identity of the toxic component of peritoneal fluid resulting from strangulation obstruction has long been debated There is considerable evidence which indicates that bacteria are responsible for the lethal characteristics of this material[2] The bacterial flora of the upper gastrointestinal tract is relatively low in comparison to the ileum and colon It was therefore elected to compare the relative toxicity of fluid resulting from strangulation obstruction of various segments

METHOD

Fifty two adult mongrel dogs were used

Donor Animals. All donor animals were anesthetized with intravenous

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* From the Department of Surgery, University of Mississippi Medical Center, Jackson
Supported by National Institute of Health Grant RG-4745

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METHOD

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Donor Animals. All donor animals were anesthetized with intravenous

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pentobarbital (nembutal) Utilizing sterile technique the abdominal cavity was opened through the midline After strangulation of the stomach (4 dogs) duodenum (2 dogs) jejunum (2 dogs) ileum (2 dogs) and colon (2 dogs) the abdominal wound was closed in layers The animals were placed in their cages for observation The accumulated peritoneal fluid was collected 36 hours later or at the time of death in those animals which failed to survive this period

Stomach The stomach was mobilized by division and ligation of the gastrocolic and gastrohepatic ligaments An umbilical tape tie was placed firmly around the upper portion of the stomach so that all blood vessels were occluded The right gastroepiploic artery was then dissected free in *the region of the pylorus An umbilical tape tie was placed around the lower* portion of the stomach so as to occlude all blood vessels except the right gastroepiploic artery This resulted in a closed segment of strangulated stomach (Fig 1)

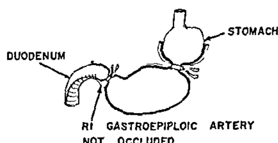


Fig 1 Method used to strangulate the stomach

Intestine The method used to strangulate the intestine has been previously described [1] Briefly this consisted of ligation of all venous drainage to a 10 cm closed loop of bowel which had been obstructed by means of two umbilical tape ties

Recipient Animals A small amount of sodium pentothal was administered intravenously to most these animals following which the strangulation fluid was injected directly into the peritoneal cavity They were observed for 24 hours

Group 1 A—(5 dogs) These animals received an intraperitoneal injection of peritoneal fluid resulting from strangulation of the stomach of two donor animals The dose was 3 cc /kg White blood cell counts were carried out upon 4 of these dogs before and after exposure to the fluid B—(10 dogs) An intraperitoneal injection of fluid resulting from strangulation of the *stomach of two donor animals was administered Five of the animals were* given 3 cc /kg while the remainder received 2 cc /kg

Group 2 (5 dogs) These animals received an intraperitoneal injection of fluid which resulted from strangulation of the duodenum in the amount of 3 cc /kg

Group 3 (5 dogs) An injection of fluid which resulted from strangulation of the jejunum was given to these animals in the amount of 3 cc /kg

Group 4 (5 dogs) These animals were given peritoneal fluid which resulted from strangulation of the ileum of donor animals The dose was 3 cc /kg

Group 5 (10 dogs) An injection of peritoneal fluid resulting from

strangulation of the colon of two donor animals was administered. Five of the animals were given 3 cc./kg. The remainder of this group received 2 cc./kg. and white blood cell counts were done upon 4 of these animals.

RESULTS

Donor Animals. All animals which sustained strangulation of the colon were dead before the end of 36 hours. This was also true for animals which experienced strangulation of the ileum. Only one of the 4 animals with strangulation of the stomach failed to survive this period. The remainder of the donor animals were sacrificed. The gross appearance of the peritoneal fluid was similar in all animals. The only outstanding physical difference in the fluid which was recovered from the various animals related to the odor. The material resulting from strangulation of the ileum and colon had a strong foul odor while most of the remainder of the fluid varied from no odor to that of fresh blood.

Recipient Animals. Unanesthetized dogs experienced severe pain when injected with fluid from the colon or the ileum while the fluid from the upper gastrointestinal tract was well tolerated by most animals.

Group 1. A—All 5 animals recovered without ill effect. The mean white blood cell count was 11,165 before injection. This value dropped to 8,850 after exposure to the fluid. B—The 5 animals which were given 3 cc./kg. failed to survive for 24 hours. When the dose of fluid was decreased to 2 cc./kg. there were 3 of the 5 animals alive at 24 hours.

Group 2. Four of the 5 animals survived which received fluid resulting from strangulation of the duodenum.

Group 3. Three of the 5 animals survived following exposure to fluid resulting from strangulation of the jejunum.

Group 4. All animals which received ileal fluid were dead within 24 hours (Table 1).

Group 5. None of the animals survived following injection of colon fluid in the amount of 3 cc./kg. The same results were obtained when the dose was decreased to 2 cc./kg. The mean white blood cell count of 4 of the latter

Table 1. Results Following Injection of Peritoneal Fluid Resulting from Strangulation of Various Segments of the Gastrointestinal Tract

GROUP	ORGAN STRANGULATED	AMOUNT OF FLUID INJECTED (CC./KG.)	NO. ANIMALS INJECTED	SURVIVORS
	Stomach	3	5	0
1 A	Stomach	3	5	3
1 B	Stomach	2		4
	Duodenum	3	5	3
2	Jejunum	3	5	0
3	Ileum	3	5	0
4	Colon	3	5	0
5	Colon	2	5	

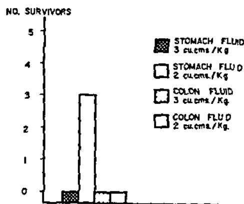


Fig 2 Results following intraperitoneal injection of various doses of peritoneal fluid resulting from strangulation of the stomach and colon (5 dogs in each group)

animals was 12 800 before injection This value dropped to 3 800 four hours after exposure

SUMMARY AND CONCLUSIONS

Peritoneal fluid resulting from strangulation of the stomach is not always lethal when the dose is 3 cc /kg When this amount of fluid was found to be lethal reduction of the dose to 2 cc /kg resulted in survival of 3 of 5 animals When the dose of fluid resulting from strangulation of the colon was reduced to 2 cc /kg all animals died The greater toxicity of colon fluid was also reflected in a more severe leukopenia following its administration It is concluded that the toxicity of peritoneal fluid resulting from strangulation increases from the upper to the lower gastrointestinal tract

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A QUANTITATIVE EVALUATION OF THE PERMEABILITY OF WET SURGICAL DRAPES TO STAPHYLOCOCCUS AUREUS *

KARL E KARLSON WILLIAM RILEY AND CLARENCE DENNIS

The increasing incidence of antibiotic resistance to staphylococci emphasizes the necessity for strict asepsis during surgery Permeability of two layers of damp surgical linen to staphylococci has been demonstrated Dry linen is resistant to the passage of these bacteria[1, 2] It is the purpose of this study to demonstrate the permeability of various thicknesses of wet surgical linen and other materials to staphylococcus aureus

METHOD

The sterile dry material to be tested was fastened over the end of a glass tube with two ties of 00 silk (Fig 1) One milliliter of a culture containing a known number of staphylococcus aureus organisms determined by dilution

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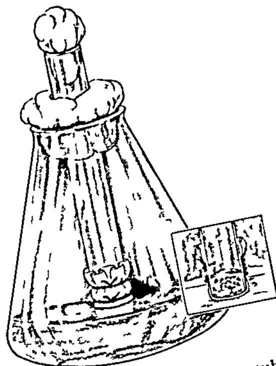


Fig 1 The inner tube with test material tied over the end is immersed 3 mm into sterile water The bacterial culture is deposited on the test material inside the tube

technique, was placed on the cloth surface facing the inside of the tube The end of the tube holding the dry drape with the organisms on the inside surface was the immersed 3 mm beneath the surface of 19 ml of sterile water The bottom tie does not touch the water After one hour, the number of organisms in the water was determined by dilution technique The results are expressed as the percentage of organisms which are subsequently recovered from the water

Two, 3, and 4 layers of ordinary muslin surgical drape have been tested In addition, an especially woven fine water repellent material,† a multi layered paper incontinence pad,†† a multi layered paper plus plastic film pad,††† and rubber dam sheeting†††† have also been tested

The permeability of these various materials to heat was evaluated by placing an insulated water container with an open top in a 66 2°F constant temperature environment and fastening the test material over the top of the vessel Water at 100 4°F was placed in the insulated vessel and the temperature of the water was recorded over the course of subsequent hours as heat loss from the water occurred The heat loss from the water is interpreted to indicate the permeability of the drape material to heat under these conditions

RESULTS

The percentage of organisms found to have migrated through each test material under the conditions described is tabulated in Table 1 Thirty per cent of the organisms placed on the dry side of two layers of muslin drape were recovered from the water into which the material was immersed When 3 layers of this drape were used, the recovery dropped to 0 5% One

† Bleached broadcloth treated with Zelan a durable water repellent
†† Under pads Ipco Institutional Products Corp
††† Chux Johnson and Johnson
†††† 0 011 inch thickness

Table 1 Bacterial Permeability of Wet Surgical Drapes Expressed as Per Cent of Original Culture

MATERIAL TESTED	n	% RECOVERY MEAN	S D
Ordinary drape			
2 layers	9	30 0	11.2
3 layers	13	0 5	0.2
4 layers	12	0.2	0 1
Finely woven drape			
1 layer	12	3 1	3 6
2 layers	13	0	
Paper pad	13	0	
Paper pad with plastic	11	0	
Rubber sheet	11	0	

layer of finely woven drape permitted passage of only 3 1% of the original culture Two layers of the finely woven water repellent drape paper incontinence pads and rubber sheet completely prevented passage of organisms under these conditions The permeability of each material to heat is reflected by the rate of decline of the water temperature with that material (Fig 2) These results indicate that the heat loss through the drape was inversely proportional to effectiveness as a bacterial barrier Of the completely effective bacterial barriers rubber sheet allows most heat transmission

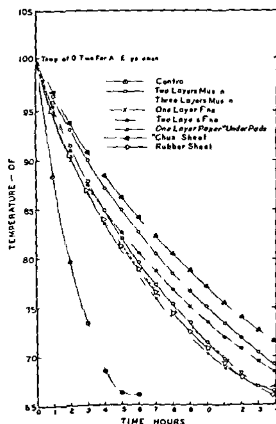


Fig 2 Heat loss from water bath covered with test material

SUMMARY

These studies indicate that two layers of wet surgical linen are unlikely to prevent passage of bacteria to the surgical field Three layers offer substantially greater security and the other materials tested are significantly more resistant to passage of bacteria Under these conditions, two layers of the finely woven water repellent material or the rubber sheeting offered bacterial impermeability with greatest heat transfer Sterile water was applied to the surface opposite to that to which the bacteria were applied, an experimental situation which reflects the clinical situation in which sterile water is usually spilled on the clean sterile surface and bacteria would migrate from the opposite side of the drape or gown Therefore, the relative effectiveness of the various combinations probably has direct clinical application The use of drapes which are relatively impermeable to the passage of bacteria when they are wet will diminish the likelihood of contamination of the surgical field from this source Special attention should be paid particularly to areas which are most likely to become wet during the operative procedure

The authors express their appreciation to Mr S G McCampbell and to Mr Sherman Converse of the Graniteville Company, Graniteville South Carolina who made the fabric available to us for evaluation

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Tumors and Tissue Transplantation

THE GROWTH OF HUMAN TUMOR CELLS IN *TISSUE CULTURE* *

GEORGE E MOORE DAVID T MOUNT AND ANNE C WENDT

Attempts to grow human tumors in tissue culture have been reported by numerous investigators Less frequently tissue culture has been employed as an adjunct method of diagnosis[2,5,9,10,13] This paper describes the utilization of tissue culture methods in further attempts to identify tumor cells in the blood[7] Confirmation of the viability of such cells further morphological identification biologic studies of their nutritional requirements and host tumor relationships were additional goals

METHOD

The infinite variety of glassware which has been proposed for tissue culture suggests that no one type has a great advantage over others These experiments utilized prescription bottles (3 oz) for stock cultures T flasks for culturing large cell samples Leighton tubes for small cell samples and petri dishes using the feeder cell technique described by Puck[11,12]

An inexpensive incubator suffices for most purposes It is advantageous to have a second small standby incubator with duplicate samples of precious cultures

Stock media are available from commercial sources Those used in these studies included #199[8] Eagles medium[3] and a modification of #199 to simulate that of Puck[6] Of seemingly greater importance than the basic medium is the kind of protein supplement There are advocates of chick embryo extract horse serum human serum calf serum and fetal calf serum For example Sano[13] who reported the culture of 400 human tissue specimens advocated the use of a combination of mouse embryo extract and autogenous fasting serum He further stated that different plasmas or serums provoked varying morphology of the cultured cells

Blood samples from peripheral veins of patients with advanced malignancy and from veins directly draining tumor sites were treated with fibrinogen heparin solution and the red cells removed after sedimenting The buffy coat was recovered from the plasma by centrifugation resuspended in a few drops of plasma or in Medium 199 and added to the cultures

It was realized that the successful culture of single tumor cells by orthodox cultures would be difficult therefore modifications of the feeder cell technique described by Puck[11,12] were investigated Briefly a suspension of well established tissue culture cells is prepared by adding 0 20% trypsin to a stock culture The cells are subjected to 3000 to 5000 r which abolishes their

* From the Departments of Surgery and Experimental Pathology Roswell Park Memorial Institute Buffalo N Y Supported by grants from the Dorothy H and Lewis Rosenstiel Foundation and the Damon Runyon Memorial Fund for Cancer Research Inc

ability to divide, yet does not kill them. A suitable number of such cells (100,000) are added to fresh media in a petri dish (60 mm.). The cells settle out and become attached to the glass (In our studies a round coverslip which exactly fitted the bottom was used so that better preparations for microscopic examination could be prepared.)

The use of fetal calf serum markedly increased the spread of the feeder cells and their attachment to the coverslip.[4] The medium used most frequently consisted of #199 to which was added 10% of calf or fetal calf serum.

The buffy coat cell suspensions were added to the medium and they settled out on the coverslip on and in between the feeder cells. This intimate physical contact apparently provides nutrients which are not contained in the medium. The culture in petri dishes were kept in a humidified 5% CO_2 atmosphere for the maintenance of proper acid-base equilibrium. The coverslips were removed in 10 to 16 days, the cells fixed in Bouin's solution and stained with Harris hematoxylin.

The cultured human cancer cells, if any, were either scattered or in compact colonies which apparently initiated from a single cell. Occasionally the cells migrated out from a clotted fragment. Often the scattered cells were intimately associated with the underlying feeder cells giving evidence of the latter's importance (Fig. 1 and 2).



Fig 1 Epithelial cells growing in contact with underlying stellate sarcoma 180 feeder cells Breast carcinoma

Fig 2 Culture of a buffy coat suspension from peripheral blood sample. Morphological studies revealed tumor cells in the blood of this patient with advanced breast cancer. Diffuse colonies of epithelial cells growing over feeder cells (Hela).

Tumors and Tissue Transplantation

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Although his record of successful cultures for diagnostic purposes was excellent the observations were limited to a 3 to 10 day period No sustained cultures are mentioned It is noteworthy that in several instances cultures of pleural effusions revealed tumor cells which had not been demonstrated by histologic and smear techniques

Coman[2] reported the culture of ascitic and pleural effusions In most instances a solid plug of centrifuged cells was used for culture He observed the growth of a colony from a single cell The ease of culturing a clump of cells rather than single cells was obvious from their use of clots to hold the cells together

Berman *et al*[1] have reported the culture of malignant human cells from bone marrow ascitic and pleural fluid and from peripheral blood Unfortunately the authors do not state whether the successful culture from peripheral blood was from a cancer patient Morphologic studies of peripheral blood in this laboratory have revealed occasional reticulum cells endothelial cells and squamous epithelial fragments from the insertion of the hypodermic needle—any of these cells might survive in tissue culture This observation again emphasizes that cell culture *in vitro* is not infallible evidence of the presence of malignant cells and that further histologic or biologic testing is required

CONCLUSION

1 A few successful cultures of epithelioid cells have been grown from blood samples bone marrow biopsies and pleural and peritoneal effusions of patients with malignancy

2 The growth of isolated cancer cells is difficult Further application of the feeder cell principle or more adequate medium should enable a greater number of successful cultures The identification of such growth factors may be of fundamental importance

3 The use of tissue culture as a diagnostic adjuvant for the study of tumor cells in the blood and in pleural and peritoneal fluids should be supplemented by subsequent biologic and histologic confirmation

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THE VIABILITY OF CIRCULATING CANCER CELLS IN EXPERIMENTAL CANCER*

OLGA JONASSON

During the last two or three decades several groups of workers have reported the demonstration of cancer cells in the circulating blood of patients with malignant disease[1,2,3,4,5] but the true significance of these cells is not known. We thought it desirable to conduct animal experiments hoping to obtain information concerning the viability of circulating cancer cells. Accordingly we obtained three experimental tumors which metastasize freely and planned an experiment injecting blood from one animal with an advanced tumor into a normal animal.

METHOD

Experimental Animals and Tumors. Initial studies were made using the T150 Lewis bladder carcinoma in C 57 black line mice. This tumor is known to metastasize via the blood stream to the lungs within approximately 14 days after inoculation.[6] The T241 fibrosarcoma, which also metastasizes fairly uniformly via the blood stream, was also used in the latter parts of the study. In addition, we examined the blood of a few white rats with the Walker 256 carcinosarcoma; in our laboratory, this tumor frequently metastasizes to the mediastinal nodes and occasionally to the lungs after subcutaneous implantation.

Collection of Blood Samples. Blood samples were initially obtained from animals bearing far advanced tumors (usually on the 20 to 25th day after inoculation of tumor) by intracardiac puncture. However, this technique afforded great risk of contamination of the blood sample by the numerous large pulmonary metastases which were nearly always present in the mice or by the mediastinal node metastases seen often in the rats. Therefore, most of the blood samples from mice have been obtained by amputation of a hind leg or small portions of the tail; nearly 1.0 cc. of blood is uniformly available using this method. Blood samples from rats are obtained by cannulation of the aorta through a posterior retroperitoneal approach; 8 to 9 cc. of blood can be obtained from each rat.

Isolation of Cancer Cells. Cancer cells were isolated from these blood samples using in essence the technique of Roberts and associates.[5] To each heparinized blood sample bovine fibrinogen was added, resulting in rapid sedimentation of the erythrocytes, leaving the leukocytes and tumor cells suspended in the plasma. Using Wintrobe hematocrit tubes, the supernatant plasma was then layered over isotonic bovine albumin with a specific gravity of 1.065; an interface was formed between the plasma with the suspended cells and the albumin. The tubes were then centrifuged, causing the polymorphonuclear leukocytes and many lymphocytes to pass through the interface to the bottom of the tube, while the tumor cells together with some lymphocytes, mesothelial cells and other mononuclear cells were arrested at the plasma-albumin interface. The contents of this interface were aspirated

* From the Department of Surgery, University of Illinois College of Medicine, Chicago.
Supported by a grant from the American Cancer Society, Illinois Division.

'd, smeared onto slides, fixed and stained by the Papanicolaou tech · Cancer cells identical to those seen in direct smears of the tumor identified in these slides

RESULTS

blood samples from nine rats with very large Walker tumors, cancer were found in very scanty numbers (3 to 4 per sample) in three animals low figure may perhaps be accounted for by the fact that the Walker r does not as a rule, metastasize via the blood stream but is primarily l growth with some lymphatic extension

ncer cells have been found in the blood as early as 10 days following taneous inoculation of the T150 tumor (Fig 1) In 34 blood samples from mice with the T150 tumor at all stages of growth, early to lvanced, cancer cells were identified in 19 samples As the tumors pro d *in size over 18 days, all blood samples prepared correctly contained* r cells, identical to those seen in direct smears of the tumor The ils with the largest tumors also had the greatest number of cancer n the blood samples Some samples contained several hundred cancer

eral large and many smaller types of cancer cells have been identified ood samples of mice with the T241 fibrosarcoma (Fig 2) Cancer cells been identified in all blood samples taken from mice bearing this r in a far advanced state i e, at least 20 days after inoculation However, ood samples taken from each of 5 mice every day from the first to the day after inoculation of the tumor into a hind leg, have failed to reveal r cells, one sample taken on the eleventh day does have a very few r cells present

enty mice with far advanced T150 tumors were bled with sterile utions One half of each sample was saved for cytologic demonstration ncer cells each sample contained such cells The remainder of each e was injected transthoracically into the heart of a normal recipient e Nine mice died as a result of the injection Of the remaining 11 receiving intracardiac blood (with much unavoidable intrapleural seven developed intrapleural tumors with gross tumor also in the lungs



Cancer cells from the blood stream *ouse with T150 tumor (1100×)*



Fig 2 Cancer cells from the blood stream of a mouse with T241 tumor (1900×)



Fig 3 A Tissue section of the original T150 tumor (125×) B. Tissue section of intrathoracic tumor after injection of blood containing tumor cells (125×)

Histologic section of this intrapleural tumor proved to be identical to the histologic sections of the original T150 tumor (Fig. 3).

On four occasions we have pooled blood samples from 10 to 50 mice with the T241 tumor. The pooled samples were prepared for isolation of cancer cells as described above, using sterile technique and solutions throughout, and the isolated cancer cells were injected subcutaneously into normal mice. Of these 4 inoculations, one tumor growth was obtained despite the presence of identifiable cancer cells in each of the samples

We have also attempted to transplant the Walker 256 tumor by injection of blood samples obtained from the aorta of a tumor-bearing rat. Two subcutaneous inoculations into normal animals have been performed, of whole blood from tumor bearing rats The first injection was of a heparinized blood sample, and no tumor growth was obtained The second inoculation was of a clotted blood sample which was implanted under the skin. A rapidly growing tumor developed which was grossly similar to other Walker 256 tumors, but which, unfortunately, was not examined histologically.

COMMENT

The identification of cancer cells in the peripheral blood of patients with cancer, and in particular the apparent direct relationship of these cancer cells in the blood to surgical manipulation of the tumor, as has been observed on several occasions by Roberts and associates,[5] makes it imperative to understand the true significance of the cancer cells which can be isolated in the blood stream This study demonstrates that, at least in experimental animals, these circulating cancer cells are viable and capable of producing new growth of tumor when injected into a normal animal Presumably these cells possess the same potentialities in the original host animals.

SUMMARY

Cancer cells have been identified in the circulating blood of mice with the T150 and the T241 tumors, and in rats with the Walker 256 carcinosarcoma Injection of whole blood or of cancer cells isolated from the blood of

animals with far advanced tumors into normal animals has resulted in tumor growths in the normal animals

It is concluded that these isolated cancer cells in experimental animals are viable and capable of producing new growth of tumor

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A NEW TECHNIQUE FOR THE DETECTION OF TUMOR CELLS IN THE BLOOD STREAM AND ITS APPLICATION TO THE STUDY OF THE DISSEMINATION OF CANCER *

JOHN F POTTER AND RICHARD A MALMGREN

The patterns of the metastatic spread of cancer as observed at postmortem examination have been well known for many years These descriptions therefore reflect only the end result of a long standing disease process which has resulted in the death of the patient As distinguished from these anatomic studies there has been little investigation of the physiologic aspect of the hematogenous dissemination of cancer

It is apparent that any study of the dynamics of metastasis must begin with the detection of the cancer cell in the circulating blood Thus in a blood specimen a perhaps single cancer cell must be isolated and identified from many millions of erythrocytes and leukocytes

A method[1] for detecting tumor cells in the blood has been developed in this laboratory and utilizes the principles of hemolysis and leukolysis by Streptolysin O an enzyme elaborated by the streptococcus With the red cells and polymorphonuclear leukocytes thus destroyed enzymatically the resultant suspension of tumor cells and lymphocytes is filtered through a millipore filter This filter is a membrane of cellulose ester which is 150 micra thick has a constant pore size of 5 micra and becomes transparent when wet with a liquid of the same refracture index The cells are collected on this membrane and are stained directly on it

METHOD

A blood specimen is placed in a siliconized centrifuge tube and is spun at 300 x g for 25 minutes The supernatant plasma is then removed by

* From the Surgery and Pathologic Anatomy Branches of the National Cancer Institute National Institutes of Health Public Health Service U S Department of Health Education and Welfare Bethesda Md

aspiration and discarded The residual cell mass is washed with physiologic saline and is again centrifuged The supernatant saline is aspirated and then a second washing is performed The removal of the plasma and the washings of the cell pack are essential for the removal of anti streptolysin which occurs naturally in plasma and which inhibits the hemolytic process

Following this the packed cells are resuspended in normal saline and Streptolysin O in saline solution is added The amount of Streptolysin used is 30 mg per cc of blood in the original specimen This suspension is then incubated at 39°C for 10 minutes producing lysis of erythrocytes and polymorphs

At the end of the incubation period to remove proteinaceous debris 2 cc of 5% glycerine solution is added to the cell suspension and centrifugation for 15 min at 700 × g is performed The supernatant fluid is removed, saline is added and the cells are collected by filtering the suspension through a millipore filter The filter membrane is fixed in 5% acetic acid alcohol solution and then in 10% formalin The filter is stained by the Papanicolaou technique and the filters are mounted on glass slides for cytologic screening

In this laboratory, this technique has proven quite satisfactory The slides are easily and rapidly prepared The cells both lymphocytes and tumor cells are spread evenly over the filter membrane without crowding These factors have facilitated screening and identification of tumor cells Finally, the technique is sensitive and quantitative In artificial specimens prepared by suspending a counted number of tumor cells in blood complete recovery of all the cells after processing has been accomplished on the filter membrane

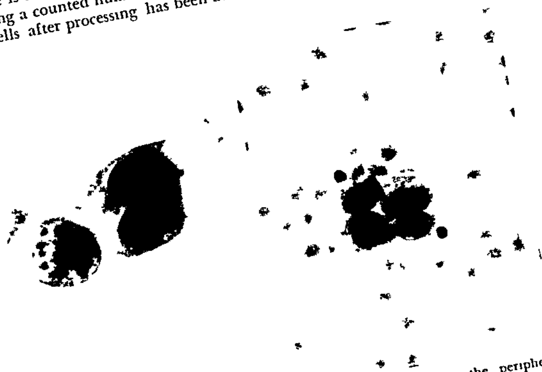


Fig 1 Cancer cells obtained preoperatively from the peripheral blood of a patient with adenocarcinoma of the uterus Has not developed metastasis in period of observation (8 months)

Fig 2 Cancer cells from the peripheral blood of a patient with metastatic malignant melanoma Cells easily distinguished from background of lymphocytes

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Fig 2 Cancer cells from the peripheral blood of a patient with metastatic malignant *melanoma* Cells easily distinguished from background of lymphocytes

Thus quantification of the number of tumor cells in a given amount of blood is possible

RESULTS

Blood specimens have been obtained from cancer patients both from the peripheral venous system and from the local veins draining the tumor One hundred eighty four specimens have been analyzed and the data recorded in Tables 1, 2 and 3

Table 1 Local Venous Blood

NO OF SPECIMENS	TYPE	POSITIVE	SUSPICIOUS	NEGATIVE
35		54%	17%	29%
9	Adenocarcinoma	56%	11%	33%
18	Epidermoid	44%	28%	28%
8	Other (Melanoma Sarcoma Choriocarcinoma)	75%	0%	25%

Table 2 Peripheral Venous Blood

NO OF SPECIMENS	TYPE	POSITIVE	SUSPICIOUS	NEGATIVE
149		24%	10%	66%
58	Adenocarcinoma	21%	12%	67%
23	Epidermoid	13%	9%	78%
68	Other	29%	9%	62%

Table 3 Summary of Peripheral and Local Specimens

NO OF SPECIMENS	POSITIVE	SUSPICIOUS	NEGATIVE
184	29%	11%	60%

DISCUSSION

In an attempt to elucidate the physiology of metastasis, a study is in progress to correlate the presence of cancer cells in the blood with the biologic behavior of the tumor and the patient's clinical course The data obtained are still of a preliminary nature but certain impressions have been reached

The incidence of tumor cells in the blood is, as might be expected much higher in veins draining the tumor than in peripheral blood The incidence of positive cases by tumor type correlates well with the known clinical characteristics of the specific tumor Thus patients with sarcomas and melanomas were more frequently positive than, successively, patients with adenocarcinoma and epidermoid cancer

Additional impressions have been gained by the study of individual cancer cases. Although there is a direct relationship between the stage of the patient's disease and the recovery of positive cells, nevertheless, in certain cases cancer cells have been found in the local blood of patients with very early clinical cancer. Some of these patients furthermore did not have metastases at the time of surgery and have not subsequently developed them. This supports the impression that not all cancer cells which embolize produce metastasis. It is conjectured that certain patients possess some type of intrinsic host resistance which destroys the detached cancer cell.

SUMMARY

A technique is described which allows detection and quantification of cancer cells in the blood. Preliminary results are reported. Studies are being undertaken to elucidate the clinical significance of the cancer cell in the circulating blood stream.

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ANTITUMOR EFFECTS OF HOMOLOGOUS IMMUNE SERUM, IMMUNE LYMPHOCYTES AND NITROGEN MUSTARD AGAINST A RAT LYMPHOMA*

FREDERICK W. PRESTON, ELIZABETH JANE JACKSON, GEORGE C. HENEGAR AND ROBERT SCHREK

There are two elements involved in the mechanism of homologous tumor immunity in rodents: 1) a humoral element which depends upon circulating cytotoxins or antibodies in the serum; and 2) a cellular element which depends upon the tumor inhibiting properties of lymphocytes.

At the 1954 Forum we reported the cytotoxic effects of an homologous immune serum prepared against the Bagg rat lymphosarcoma.[1] The s…

Because the *in vitro* cytotoxic effects of this immune serum seemed striking, experiments were undertaken to test its effect against viable malignant cells in the intact animal. The tumor inhibiting properties of immune lymphocytes were studied concurrently.

In the experiments reported in this paper, Bagg's lymphosarcoma was injected intraperitoneally to simulate as nearly as possible the dissemination

* From the Departments of Surgery, Veterans Administration Research Hospital and the Northwestern University Medical School, Chicago. Aided by grants from the United Fund of Northfield, Illinois, the Frederick Augustus Preston Memorial Fund for Cancer Research, the Illinois Division of the American Cancer Society, and research grant CY 4049 from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

of tumor cells that occurs during the removal of visceral neoplasms in patients The protective action of immune serum immune lymphocytes, and mechlorethamine hydrochloride (nitrogen mustard, HN_2) against these implants was evaluated This report differs from a previous report from our laboratory in that the experiments were done with inocula containing fewer tumor cells [4] Nitrogen mustard was used in these experiments because this tumor has been shown to be sensitive to this drug

METHOD

The Bagg's lymphosarcoma used in these experiments was described by Goldfeder [5] It was maintained in our laboratory by subcutaneous inoculation into random bred albino rats of the Wistar strain The histologic and growth characteristics of this neoplasm have changed only slightly during 90 consecutive transplants The last 50 transplants grew in all of the rats In 83% of these animals, growth was progressive resulting in death in 23 days or less In 17% of the animals growth of the tumor was followed by regression and survival of the animal Rats with regressed tumors are immune to subsequent transplants of the tumor

Preparation of Malignant Lymphocytes. Suspensions of Bagg's tumor cells in human serum were prepared for intraperitoneal inoculation by chopping up an actively growing tumor excised from a subcutaneous site The suspensions were filtered through an 80 mesh Monel wire screen Sterile technique was used, and bacterial cultures of the cell suspensions done with each transplant showed no bacterial growth The tumor cells were counted by the method of unstained cell counts [6] and diluted with human serum so that 7,000 to 9,000 viable cells were contained in 0 1 ml of suspension This was the dose range of malignant cells used in these experiments The dose used in the experiments that we previously reported varied from 32,000 to 65,000 viable cells per 0 1 ml of suspension [4]

Young adult male random bred Wistar rats weighing 100 to 150 gm were used for the transplants They were kept in cages containing 10 to 20 rats and fed standard rations The animals received intraperitoneal injections of malignant cells through a 22 gauge needle Following the injections, the abdomens were massaged to disperse the cells through the peritoneal surfaces

Preparation of the Immune Serum. Rats with regressed Bagg s tumors were used as donors of the immune serum The immunity of each rat was challenged and enhanced by an inoculation of tumor within 35 days prior to the time of preparation of the immune serum In each case the challenge dose failed to take,' indicating that the rat was immune Blood was obtained by cardiac puncture from these animals under anesthesia The serum was obtained by centrifugation

Preparation of Immune Lymphocytes. The thymus glands and spleens were excised from the same rats used as donors for the immune serum These organs were chopped up and the cells washed through an 80 mesh Monel wire screen with small amounts of human serum The number of viable lymphocytes obtained was determined by the method of unstained cell counts [6] To test the antitumor effect of immune lymphocytes, 9 rats were given $2 4 \times 10^6$ viable lymphocytes and a second group of 13 rats was given $6 2 \times 10^6$ lymphocytes immediately after receiving an inoculation of Bagg lymphosarcoma A third group containing 10 rats received immune lymphocytes as

follows: 3.0×10^9 lymphocytes 24 hours after the inoculation of tumor cells, 8.5×10^9 lymphocytes 48 hours after the inoculation, and $3\,5 \times 10^9$ lymphocytes 72 hours after the inoculation.

Control Experiments. The first control group of animals received intraperitoneal lymphosarcoma cells and no subsequent treatment. Sixty animals were inoculated with 7,000 tumor cells, 24 with 8,000 and 8 with 9,000 cells. Thus, a total of 92 animals received 7,000 to 9,000 viable tumor cells. There is no difference in the effect on the animal of the number of cells in the inoculum within this range.

A second control group containing 9 rats received 7,000 viable tumor cells and immediately following was given 4.3×10^9 lymphocytes prepared in an identical manner as the immune lymphocytes except that they were obtained from normal rats previously unexposed to tumor transplants.

A third control group of 13 animals was given 8,000 viable tumor cells and immediately thereafter a suspension of minced kidney tissue prepared from immune rats in the same manner as the immune lymphocytes. The purpose of this control was to determine whether the small amounts of immune serum and human serum carried by the cell suspensions exerted an antitumor effect. The use of cell suspensions washed free of all adherent serum by saline solution was tried, but it was found to be impractical because washed cell suspensions could not be filtered readily. This control also served to determine the specificity of immune lymphocytes by determining the antitumor effect of a cell suspension prepared from another organ of a tumor-immune rat.

Control animals given tumor cells followed by normal rat serum are not included in the study because it has been shown previously that normal rat serum offers no protection against the lethal effects of this tumor.[4]

RESULTS

The antitumor effect of the immune serum, immune lymphocytes and nitrogen mustard was determined by the ability of these agents to prevent death of the animals (Table 1).

The tumor usually killed the animals between the 9th and the 15th day after inoculation. No deaths from tumor occurred after the 23rd day except for one animal treated with nitrogen mustard that died on the 24th day. Autopsy of animals dying of tumor revealed extensive malignant growth attached to the peritoneal surfaces, liver and spleen. Moderate bloody ascitic fluid was present In some animals, there was gross evidence of invasion of the retroperitoneal lymphatics. Hematogenous metastases did not occur. The postmortem appearance of animals that died of tumor in the experimental group did not differ from those that died in the control group. The median survival time of the animals that died in the experimental group was slightly longer than that of the control group.

The immune serum had a definite antitumor effect. Of 80 rats given lymphosarcoma and immediately thereafter inoculated with immune serum, 66 (83%) were alive 23 days after the inoculation (Table 1). This is to be compared with the untreated control group in which there were 92 animals. Of these, 21 (23%) survived for 23 days.

Age of the immune serum had no effect on its antitumor effect as shown in Table 2. The 23 day survival was 83% for 42 rats treated with fresh

Table 1 Effect of Serum, Lymphocytes and HN_2 on Rats Inoculated with Baggs Lymphosarcoma Cells Intraperitoneally

SUBSEQUENT TREATMENT (ALL INJECTIONS INTRAPERITONEALLY)	NO OF RATS	RATS SURVIVING 23 DAYS NUMBER	%
CONTROL GROUPS			
None	92	21	23
4 3 x 10^6 normal lymphocytes	9	0	0
Suspension of immune kidney cells	13	1	8
EXPERIMENTAL GROUPS			
2 ml immune serum †	80	66	83
2 ml fresh immune serum ††	15	11	73
2 ml fresh immune serum †††	15	11	73
2 ml fresh immune serum †††††	15	12	80
2 4 x 10^9 immune lymphocytes †	9	5	56
6 2 x 10^9 immune lymphocytes †	13	10	77
3 0 x 10^9 to 8 5 x 10^9 immune lymphocytes ††††	10	7	70
0 8 mg /kg HN_2 †	15	11	73
1 0 mg /kg HN_2 †	15	11	73
0 4 mg /kg HN_2(total dose 1.2) †††††	15	12	80

† Dose given immediately after the intraperitoneal inoculation of tumor cells

†† Dose given immediately after intraperitoneal inoculation of tumor cells and repeated 24 hrs later

ls

tumor cells

of tumor cells

Table 2 Effect of Age of Serum in Protecting Against Lethal Effect of Lymphosarcoma Cells Injected Intraperitoneally

SUBSEQUENT TREATMENT (ALL INJECTIONS INTRAPERITONEALLY)	NO OF RATS	RATS SURVIVING 23 DAYS NUMBER	%
2 ml fresh immune serum	42	35	83
2 ml immune serum (7–10 days old)	12	11	92
2 ml immune serum (21 28 days old)	26	20	77
Total	80	66	83

immune serum It was 92% for 12 rats treated with serum that was preserved at 1°C for 7 to 10 days and it was 77% for 26 animals treated with serum refrigerated for 21 to 28 days

There is some evidence that immune serum is effective against established tumors One group of 15 rats received the immune serum 24 hours after the inoculation of tumor cells Twenty three days later 11 (73%) were alive Another group of 15 rats was given immune serum 72 96 and 120 hours after receiving the tumor cells Twelve (80%) of these animals were alive 23 days later

Immune lymphocytes also exerted an anticancer effect There were 22 rats that received lymphocytes immediately after the tumor cells Of these, 15 (68%) were alive 23 days later This is in contrast to the control group that received normal lymphocytes in which there were no survivors at 23 days It can also be contrasted to the control group that received a suspension of immune kidney cells Of 13 animals one (8%) was alive at the 23 day interval

Ten rats received immune lymphocytes 24 48 and 72 hours after receiving the tumor cells Seven (70%) were alive 23 days later This group is too small to warrant a conclusion that immune lymphocytes are effective in protecting against established tumors

Nitrogen mustard was injected intraperitoneally according to three dose schedules Fifteen rats received a single dose of 0 8 mg /kg and another 15 animals received 1 0 mg /kg immediately following the intraperitoneal inoculation of tumor cells In both groups 11 animals (73%) survived for 23 days Another group of 15 animals received 0 4 mg /kg of nitrogen mustard 72 96 and 120 hours after the tumor cells Twelve of these (80%) survived for 23 days

DISCUSSION

These experiments show that the course of malignant disease caused by implanted tumor in a susceptible animal may be altered by homologous immune serum and homologous immune lymphocytes Both seem to be as effective as is nitrogen mustard a cytotoxic drug to which the tumor is sensitive

In one experiment the serum was given for 3 days beginning 72 hours after the tumor was implanted A similar number of rats was given nitrogen mustard on the same dose schedule The survival of animals in these groups suggests that these agents are effective against established tumors under the conditions of these experiments

SUMMARY

The antitumor effects of an homologous immune serum and homologous immune lymphocytes were demonstrated by the ability of these agents to reduce the mortality following intraperitoneal inoculation of Bagg s lymphosarcoma in rats

Nitrogen mustard in total doses of 0 8 to 1 2 mg /kg produced a similar therapeutic effect

(The authors are indebted to Dr Anna Goldfeder who provided the Bagg s tumor)

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THE CARCINOGENIC EFFECT OF NITROGEN MUSTARD *

GERALD OWEN MCDONALD

The anticancer and biological effects produced by nitrogen mustard are similar in many respects to those produced by radiation. We would suspect, therefore, that the carcinogenic effects of nitrogen mustard also would be similar to the proved carcinogenic effects of radiation. The work of Boyland and Horning [1] has suggested that this is true when massive doses are used. However, we were concerned with the possible carcinogenic effect of nitrogen mustard when smaller, human clinical dose levels were used.

No observations have been reported of the carcinogenic effect of nitrogen mustard in the human. When Cole and associates began the clinical trials of the prophylactic or adjuvant treatment of cancer in March, 1956, we were concerned with the possible late carcinogenic effect of the drug. In addition to receiving the agent at the time of surgery, the patient was recalled at intervals of 4 months for subsequent courses of therapy. Knowing that radiation can cause cancer and that very large doses of nitrogen mustard have been reported as causing cancer in the mouse, we felt it was important to determine if smaller, clinical doses of nitrogen mustard, given repeatedly, could cause cancer in the experimental animal.

METHOD

Repeated clinical dosage levels of 0.4 mg. of nitrogen mustard/kg. of body weight were administered to a group of 180 female albino rats (Holtzman), Group 1. Sixty of these (Group 1-A) received the mustard subcutaneously in their right flank; 120 (Group 1-B) received the mustard intraperitoneally. The nitrogen mustard was diluted in physiological saline so that 0.1 mg. of mustard was contained in 10 ml. of solution. In this concentration the mustard does not exert a vesicant action. The injections were given at intervals of 4 weeks over a 9 month period for a total of 10 courses or treatments of the drug.

A second group of 180 rats (Group 2) received repeated injections of 1.0 mg. of nitrogen mustard/kg. of body weight. These animals were divided in the same manner as Group 1, 60 animals (Group 2-A) receiving the mustard subcutaneously and 120 (Group 2-B) receiving the drug via intraperitoneal injection. The nitrogen mustard was diluted in the same manner and was given at the same intervals over a 9 month period.

A third group (Group 3) of 272 animals received no drug and served as control animals Groups 4 and 5 consisted of 60 animals each and received only one initial injection of nitrogen mustard, Group 4 receiving 0.4 mg./kg of body weight and Group 5, 1.0 mg./kg. of body weight.

The animals were examined weekly for evidence of tumor development. Those animals which developed tumors were photographed and then observed to determine the growth characteristics of the tumor. All animals were sacrificed and microscopic sections of the tumors were prepared. Those tumors which appeared to be rapidly growing were transplanted into other

* [illegible] ...ois, Chicago Supported in part by ...SPH Grant CY 3482 from the N.C.I.

animals. These tumors also were maintained in tissue culture preparations.

RESULTS

The incidence of tumor development in each group is shown in Table 1. Tumors developed in 15 of the 180 animals which received multiple doses

Table 1. Tumor Development

	NUMBER OF ANIMALS	TUMORS NUMBER	TUMORS PER CENT
1. 0.4 mg./kg.—multiple dose			
A—Subcutaneous	60	5	8.3
B—Intraperitoneal	120	7	5.8
Total	180	12	6.7
2. 1.0 mg./kg.—multiple dose			
A—Subcutaneous	60	2	3.3
B—Intraperitoneal	120	13	10.8
Total	180	15	8.3
3. 0.4 mg./kg.—single dose	60	8	11.7
4. 1.0 mg./kg.—single dose	60	4	6.7
Total—Receiving nitrogen mustard	480	39	8.1
5. Control	273	17	6.2

of 0.1 mg./kg. of body weight of nitrogen mustard, an incidence of 8.3%. Tumors developed in 12 of the 180 animals in Group 2, the multiple injection 0.4 mg. group, an incidence of 6.7%. In the control group of 272 animals, 17 developed tumors, an incidence of 6.2%. Thus there was really no significant difference in the incidence of tumor development in these 3 groups of animals, the control group developing tumors in approximately the same incidence as those animals receiving both large and human clinical dosage levels of nitrogen mustard.

There was, however, a difference in the time of development of tumors in these various groups. The animals receiving multiple doses of nitrogen mustard began developing tumors by the sixth month following initiation of the injections. By the end of the middle of the tenth month after the injections were begun, tumors had appeared in 14 of the animals receiving multiple injections of nitrogen mustard. Five of these tumors occurred in the group receiving the human clinical dose (0.4 mg./kg.) while 9 of the tumors occurred in the animals receiving the larger (1.0 mg./kg.) dose. No tumor appeared in any of the single injection nitrogen mustard groups or in the control animals until the end of the third week of the tenth month. This was 20 weeks (4¾ months) following the appearance of tumors in the multiple dose groups. Over one half of the tumors appearing in the multiple injection group had appeared before any tumors developed in the control or single injection groups.

The microscopic appearance of these tumors varied greatly, from frank adenocarcinomas to small cell sarcomas. Some of the tumors grew very slowly, others grew rapidly. Four of the animals had tumors arising in the cervical

area All of these grew rapidly, arising and reaching a size of 4 by 4 by 4 cm within 10 to 14 days These tumors were transplanted, by means of tumor cell suspensions, both subcutaneously and intramuscularly into other rats The transplanted tumors grew rapidly for 5 to 8 days and then regressed completely leaving only small 3 by 3 mm nodules On microscopic section these nodules showed only foreign body, granulomatous changes Cell suspensions of these tumors and explants in hanging drop preparations were placed in tissue cultivation in the tissue culture laboratory They grew rapidly, forming sheets of cells, but regressed and growth ceased in 3 to 5 weeks This was not due to contamination or errors in technique as other tissues were maintained under the same conditions and techniques and continued to grow satisfactorily

DISCUSSION

Nitrogen mustard long has been used clinically in the treatment of cancer and the leukemias However, its use in cancer essentially has been palliative limited to those patients whose disease has not been amenable to surgery or has not been eradicated by surgery This has failed to allow clinical observation of the possible later carcinogenic effects of this agent, as in practically all cases the patient has failed to live long enough for observations to be made When the clinical trial of the administration of anticancer agents at the time of surgery was begun[2] we were concerned with the possible later carcinogenic effects of these powerful drugs This was exceptionally important as in this prophylactic or adjuvant treatment we were administering nitrogen mustard in the hope of increasing the survival rates in patients with carcinomas of the breast, colon, rectum, and stomach In addition we were administering repeated courses of nitrogen mustard at intervals of 4 months, changing to ThioTEPA at the end of the third course of mustard The possibility of causing the appearance of other malignancies in these patients due to the carcinogenic effects of these agents presented a real hazard We proceeded with the clinical trials of this procedure, however as we had found no evidence that nitrogen mustard in clinical dosage levels was carcinogenic Ochsner[3] had given repeated courses of nitrogen mustard over extended periods of time without observing any evidence of a carcinogenic effect The experimental animal work reported by Boyland and Horning[1] was suggestive that this drug was carcinogenic when extremely large doses were given However their control animals were sacrificed before they had reached the same age as all of the treated animals If allowed to reach this age it is conceivable that an equal number of tumors would have developed in both groups

The findings of the present investigation clearly indicate that the control group of rats had tumors develop in approximately the same incidence as did the 2 groups of animals receiving multiple injections of nitrogen mustard The findings would suggest that nitrogen mustard when given in this manner (similar to the manner we are using clinically as a surgical adjuvant) is not carcinogenic These animals received injections of the drug at monthly intervals for a total of 10 injections Injections were stopped at the end of the ninth month as the animals receiving the larger dose of mustard developed marked leukopenia and bleeding tendencies Injections were stopped at this time as it was obvious the animals would not tolerate further injections

without the loss of many of them These animals have been followed for a period of 15 months since the injections were started and are now 18 months of age It is probable that more tumors will arise during the next 12 months but we would anticipate that these results will be maintained

SUMMARY

Repeated injections of nitrogen mustard were given to 2 large groups of animals at monthly intervals for 9 months One group received repeated injections of 0 4 mg /kg of body weight while the other received 1 0 mg /kg of body weight A third group of animals received no nitrogen mustard and served as a control group The incidence of tumor development in the control animals was approximately the same as the incidence of tumor development in those animals receiving multiple doses of nitrogen mustard It appears therefore that nitrogen mustard is not carcinogenic when given to animals in a manner similar to that used clinically as a surgical adjuvant

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EXPLORATORY STUDY OF VASCULAR OCCLUSION AND PERFUSION TECHNIQUES FOR THE LOCALIZATION OF ACTION OF CYTOTOXIC DRUGS *

WILLIAM M PARKINS ROBERT G RAVDIN AND PETER F COGGINS III

Utilizing a balloon catheter † technique to occlude the distal aorta and inferior vena cava in dogs a clinically adaptable perfusion method has been devised for the preferential localization of the effect of rapidly acting cytotoxic agents such as nitrogen mustard (HN_2) †† This work has been done to evolve a method of clinical treatment for localized pelvic malignancy which is surgically inoperable and has been treated with irradiation to tolerance While we are aware of previously reported experimental techniques for the perfusion of isolated extremities and portions of the midgut [1,2] reference has not been found to work on a technique applicable to perfusion of the hypogastric circulation without requiring that the abdomen be open

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METHOD

The pelvic vascular bed and posterior extremities were isolated in 36 dogs under pentobarbital anesthesia by means of occlusive balloon catheters placed by way of the femoral vessels in the aorta distal to the inferior mesenteric artery and in the vena cava just central to its bifurcation Double lumens enabled these catheters to serve for perfusion Both femoral arteries were ligated In the human tourniquets are used

After occlusion and stabilization of the resulting central and peripheral pressures saline solutions containing nitrogen mustard and/or Evans Blue dye were perfused intraarterially with a sigma pump (Fig 1) Heparin was used either systemically or in the perfusate Samples were taken periodically from the jugular vein and from the venous effluate for determination of dye density and hematocrit

The isolated vascular bed was perfused by *1*) recirculation using a venous reservoir in a continuous extracorporeal pump circuit (Fig 1 1) 2) arterial infusion of mustard solution followed by a flushout with 0 9% saline with diversion of the venous outflow (Fig 1 2) 3) arterial infusion of the isolated bed without diverting the venous outflow (Fig *1* 3) At the completion of *the perfusion the occlusion was released the pressures allowed to equilibrate* and the catheters were withdrawn The dogs were observed daily and hematologic data were obtained at weekly intervals They were sacrificed at 2 4 and 8 weeks and tissues were subjected to microscopic examination

RESULTS

All experimental animals survived

In the recirculation procedure as indicated by dye concentrations exchange between the central circulation and the isolated bed by way of collaterals leads to equilibration within a few minutes Since this circuit did not accomplish the desired result its study was discontinued although it is not unlikely that more efficient separation can be achieved in the human

Arterial infusion at a controlled rate with diversion of the venous effluate proved the most efficient means of maintaining a high drug concentration in the isolated area with minimal loss into the general circulation (Fig 2) Due to the low viscosity of the perfusing fluid 3 or more times the estimated vol*ume of the isolated bed was perfused per minute without greatly diminishing* the arterial pressure differential between the 2 circulations This mitigated against diffusion from the isolated area into the general circulation and in fact resulted in some loss of blood volume by passage in the other direction (Fig 2)

Experiments were designed with the above technique to determine the influence of HN_2 concentration perfusion time and rate upon tissue in the perfused area and upon systemic toxicity (Table 1) As can be seen (Group 1) at a concentration of 1 mg % of HN_2 for 5 minutes at 10 ml /kg /min the animals had mild nausea and vomiting severe edema and moderate muscle damage in the pelvic area The absolute blood loss approximated 100 ml *When the concentration was diminished to 2 mg %* (1 mg /kg total body dose Group 2) there was no nausea and vomiting and edema and muscle damage in the hind quarters was negligible At a similar total dosage (1 mg / kg) with a concentration of *4* mg % for only half the perfusion time (Group

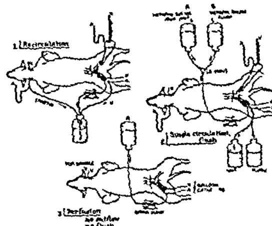


Fig 1

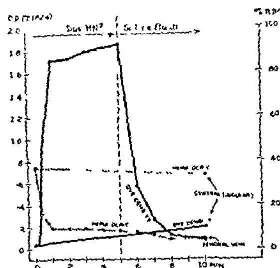


Fig 2

3), again there was no vomiting, and only +1 muscle damage and edema of the hind quarters

Table 1. Pelvic Perfusion—Single Circulation

	HN_2 PERFUSATE				EFFECTS		
GROUP	CONC. MG./100 ML.	TIME MIN	RATE ML./KG	TOTAL DOSAGE MG./KG	NAUSEA VOMITING	EDEMA	MUSCLE DAMAGE
1	4	5	10	2	+	++++	++
2	2	5	10	1	0	+	+
3	4	2.5	10	1	0	+	+
4	2	10	10	2	++	++++	++
5	4	5	5	1	0	++++	++
6	2	5	5	0.5	0	+	+

Similarly when the concentration of HN_2 was decreased to 2 mg %, and the perfusion time was doubled (10 minutes at 10 ml /kg /min, Group 4), considerable nausea and vomiting, edema, and muscle damage were noted At postmortem examination, these animals had areas of breakdown and frank necrosis of pelvic muscles Even with the perfusion rate diminished and other factors constant (total body dose 1 mg /kg), there was +4 edema and +2 muscle damage (Group 5) With the concentration of 2 mg %, perfused at the reduced rate (total dose 0 5 mg /kg) no systemic toxicity was noted (Group 6)

From these results, it may be [illegible]

[illegible] The dogs, [illegible] active, and ate on the second and [illegible] first postoperative day

Six animals were infused intraarterially without diverting the venous outflow Those receiving a total dose of 1 mg /kg at a concentration of 1 mg % vomited profusely for 48 hours, while 3 receiving 0 5 mg /kg at a

while glass spheres up to 390 to 440 μ will pass through the lungs and kidneys respectively, apparently through vascular shunts

Response to Surgery. We have seen showers of malignant cells in the circulating blood of several patients during surgical manipulation [3, 4] In these potentially "curable" patients the cancer cells disappeared from the blood stream within minutes following the removal of the primary carcinoma Two patients with cancer cells in the blood stream preoperatively were found at celiotomy to have nonresectable malignancies, and it was therefore possible to evaluate the changes which might occur in the postoperative period by drawing daily blood samples

The response of the first patient, with nonresectable sarcoma of the uterus is seen in Figure 2 Following the stress of celiotomy (biopsy of a mesenteric node) there was a decrease in the number of malignant cells, the lowest level being reached by the third day followed by a gradual return to the preoperative level by the eleventh day

A similar response was seen in the second patient, with nonresectable carcinoma of the stomach The lowest level was reached in 2 days followed by a return to the preoperative level on the fifth day [4] In both these patients cancer cells were found in every sample, the decreased number in the postoperative period being only transient It is conceivable that the transient decrease in the number of cancer cells in the blood stream during the postoperative period may represent the lodging of the cancer cells in capillary beds and their subsequent extravascular growth since it has been shown by Lewis and Cole [5] that pulmonary metastases are increased in mice after operative stress Also, Buinauskas and associates [6] have shown an increased number of "takes" of subcutaneous Walker carcinosarcoma cells following the stress of celiotomy in rats

Response to Chemotherapy. The response of a patient with nonresectable sarcoma of the uterus to chemotherapy is seen in Figure 3 Within 3 days after the administration of nitrogen mustard (0 2 mg /kg i v) no malignant cells were found in the blood stream A few malignant cells were found on the fourteenth day followed by a gradual return to pretreatment levels by the twentieth day after nitrogen mustard administration

We have previously reported [4] a decrease but no disappearance of cancer cells from the circulating blood of 2 other patients including 1 with inflammatory carcinoma of the breast and 1 with widespread neuroblastoma Sandberg and Moore [7] also reported that the administration of anticancer agents resulted in the disappearance of cancer cells from the blood of 3 patients for a 2 week period

The disappearance of cancer cells from the blood stream following 1 anticancer agent but not another would indicate which agent is effective for a *specific* tumor in a specific patient A study of this type is seen in Figure 4 This patient with nonresectable carcinoma of the stomach was given one half the usual amount of nitrogen mustard (0 2 mg /kg iv) Within 72 hours after the chemotherapy the cancer cell counts were at the lowest level followed by a gradual increase to pretreatment levels within 4 weeks The patient was then given (30 days after nitrogen mustard) ThioTEPA (0 4 mg /kg) intravenously Relatively few cancer cells were found in the blood stream prior to the administration of this second agent and none were found 4 days later Cancer cells were again present 18 days following the administration of ThioTEPA

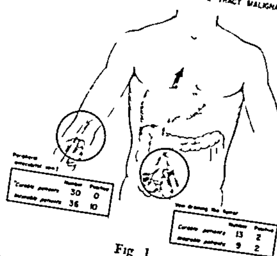


Fig 1

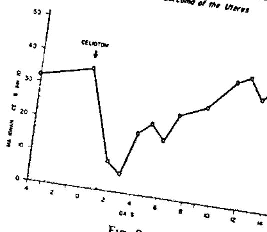


Fig 2

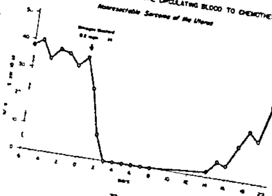


Fig 3

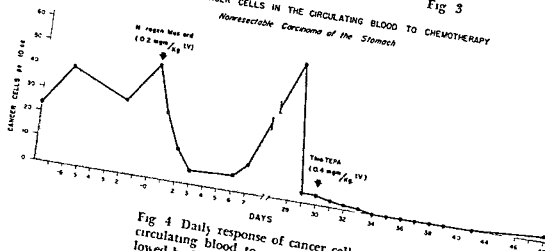


Fig 4 Daily response of cancer cells in the circulating blood to nitrogen mustard followed by ThioTEPA The disappearance of cancer cells following the administration of ThioTEPA may represent an example of in vivo sensitivity or an additive effect of the nitrogen mustard plus ThioTEPA (After Watne *et al*, Acta I U A C.)

SUMMARY

In a study of 200 cancer patients, cancer cells have been isolated from the peripheral blood (antecubital vein) in 14% of "curable" and in 33% of "incurable" patients

No cancer cells were found in the peripheral blood of 30 "curable" patients but were found in the peripheral blood in 28% of "incurable" patients with digestive tract malignancies, indicating the importance of the liver.

There was a transient decrease within 48 to 72 hours during the postoperative period in the number of cancer cells in the blood stream of 2 patients with nonresectable malignancies.

In 2 patients, no cancer cells were found in the blood stream within 72 hours following the administration of an anticancer agent with a reappearance 14 to 18 days later.

By following the serial response of cancer cells in the circulating blood to chemotherapy, it may be possible to determine *in vivo* sensitivity of a specific tumor to a specific agent in a specific patient A study of this type is described

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CONTROL OF FREE TUMOR CELL GROWTH BY TOPICAL CHEMOTHERAPEUTIC AGENTS *

COLIN G THOMAS, JR, PAUL M WEEKS AND BOBBY C BROWN

One of the characteristics of a malignant neoplasm is a loss of "adhesiveness" of its cells This loss of adhesiveness may well be responsible for the continual shedding of malignant cells into the general circulation, lymphatic channels, as well as body cavities and adjacent tissue spaces In association with the surgical attack upon neoplastic disease, this dispersion of malignant cells is probably increased [1] Wound contamination may be a particularly serious problem in those neoplasms in which there is epithelial surface involvement, such as seen in carcinoma of the oral cavity, gastrointestinal and genitourinary tract, as well as tumors encroaching into body cavities Similar contamination

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is likely in those situations requiring an open biopsy. A number of clinical observations have attested to the high incidence of neoplastic cells in the washings of such wounds as well as the ability of these cells to successfully implant.[2] Since the malignant cell appears to be much more readily destroyed when surviving as a free graft,[3] an attempt to control the spread of tumor before implantation has occurred is quite rational.

The purpose of this investigation was to study a number of tumoricidal agents which might be of some clinical value in the prevention of implantation by the free tumor cell. Because of the limitations and difficulties of evaluation of such a problem in the patient, an experimental tumor which has a known sensitivity to chemotherapeutic agents was selected.

METHOD

Mechlorethamine hydrochloride (nitrogen mustard), Clorpactin® XCB, chloramine T, compound solution of iodine (Lugol's solution) and formalin were employed as agents with Ehrlich's ascites tumor† in strain A mice as the test object. Mice of both sexes weighing between 20 and 28 gm. were employed. They were maintained on a standar[illegible]
ad[illegible]
an[illegible] inoculation and 2 ml. of the pooled ascitic fluid containing between 50 000 and 100 000 cells employed for [illegible]
[illegible] were studied. 1. The *in vitro* effect of [illegible] agent was evaluated employing 2 ml. of freshly harvested ascitic fluid and 2 ml. of the tumoricidal agent mixed by multiple inversions in a 1 ml. tuberculin syringe for approximately 15 seconds. The entire contents of the syringe were then injected intraperitoneally. 2. The *in vivo* effects of the tumoricidal agent were studied by injecting 2 ml. of the ascitic tumor intraperitoneally, then 30 to 60 minutes later 1 ml. of the agent to be tested. 3. The ability of the agent to interfere with tumor implantation in a wound was appraised by means of a 2 square centimeter wound "pocket" made on the back beneath the panniculus carnosus with the subsequent inoculation of 2 ml. of ascitic fluid. Thirty minutes later the wound was irrigated with 5 ml. of the experimental agent and the incision closed with clips.

Mice were evaluated in terms of (1) time of appearance of ascites, (2) survival time, (3) development of a subcutaneous tumor.

RESULTS

Group 1. *In vitro* mixing with subsequent injection of tumor intraperitoneally (Table I).

Varying concentrations of Clorpactin® XCB, formalin, Lugol's solution, chloramine T and nitrogen mustard were employed. Clorpactin® XCB even in concentrations of 2% was quite ineffective as a tumoricidal agent with all animals dying of ascites between the tenth and eighteenth day. Five tenths per cent formalin in saline was followed by ascites formation in all animals. Lugol's solution in dilutions of 1:10 and 1:20 was highly tumoricidal. Two per cent chloramine T resulted in 100% of the animals surviving with no evidence of ascitic tumor. All mice reacted rather vigorously to the

† A hypotetraploid strain received from T. S. Hauschka in 1956.

intraperitoneal administration of both Lugol s solution and chloramine T in contrast to there being little evidence of peritoneal irritation with the other agents studied Nitrogen mustard both in concentrations of 5 mg % and 10 mg % proved to be 100% effective in controlling subsequent tumor growth Control animals receiving distilled water died between the twelfth and eighteenth day after inoculation

Table 1

AGENT	NO OF MICE	ASCITES TUMOR	ALIVE WITHOUT TUMOR	% ALIVE
.5% Clorpactin ® †	12	12	0	0
1% Clorpactin ® †	16	15	1	7
2% Clorpactin ® †	8	8	0	0
5% formalin †	10	10	0	0
Lugol s 1 10 †	10	2	8	80
Lugol s 1 20 †	10	0	10	100
2% chloramine T †	19	0	19	100
5 mg % nitrogen mustard	14	0	14	100
10 mg % nitrogen mustard	10	0	10	100
Distilled water	15	15	0	0

† In 9% saline

Results of *in vitro* mixing of 2 ml Ehrlich ascites tumor and 2 ml of chemotherapeutic agent for 15 seconds with subsequent intraperitoneal injection in Strain A mice Evaluation on 16th day

Group 2 The results of the *in vivo* mixing of the intraperitoneally inoculated tumor was in general similar to that of the *in vitro* experiments with the exception that Clorpactin® XCB and formalin were more effective by this method (Table 2) One milliliter of Clorpactin® XCB at concentrations of 5% and 1% did not prevent tumor growth and ascites development A 2% concentration did prevent ascites development in nine tenths of the animals In 6 of these however tumor nodules developed at the site of intraperitoneal puncture Chloramine T in concentrations tolerated by the mice was ineffective Lugol s solution was ineffective in concentrations not sufficiently toxic to cause death Nitrogen mustard at a concentration of 5 mg % prevented development of the ascites tumor in 18 of 20 mice In contrast to animals receiving Clorpactin® XCB and formalin none developed nodules at the site of peritoneal puncture Control animals receiving 9% saline all developed ascites and died prior to the eighteenth day

Group 3 Pilot studies employing these agents as wound irrigants have disclosed more variable results In general however nitrogen mustard proved most efficacious (Table 3)

DISCUSSION

Efforts to minimize the number of tumor cells contaminating surgical wounds and thereby prevent tumor implantation and recurrent carcinoma

Table 2

AGENT	NO OF MICE	ASCITES TUMOR	ALIVE WITHOUT TUMOR	IMPLANT AT PUNCTURE SITE	% WELL
.5% Clorpactin ® †	10	7	3	0	30
1% Clorpactin ® †	11	6	5	2	27
2% Clorpactin ® †	10	1	9	6	30
.5% formalin †	9	0	9	2	77
.5% chloramine T †	10	All dead within 4 days without tumor			
5 mg % nitrogen mustard	18	2	16	0	89
10 mg % nitrogen mustard	10	All dead within 10 days without tumor			
9% saline	10	9	1	0	10

† In 9% saline

Results of intraperitoneal injection of 1 ml of chemotherapeutic agent 30 to 60 minutes after intraperitoneal inoculation of .2 ml ascites tumor in Strain A mice Evaluation on 16th day

Table 3

AGENT	NO OF MICE	TUMOR IN WOUND	WOUND WITHOUT TUMOR	% WELL
Control	20	20	0	0
9% saline	10	10	0	0
5% Clorpactin ® †	8	8 ††	0	0
1% Clorpactin ® †	11	7 ††	4	67
2% Clorpactin ® †	11	4 ††	7	67
Benzalkonium 1 1000	10	6	4	40
5% chloramine T	10	8	2	20
1% chloramine T	9	4 ††	5	55
2% chloramine T †	8	2 ††	6	72
5 mg % nitrogen mustard	10	2 ††	8	80
10 mg % nitrogen mustard	10	0	10	100

† In 9% saline
†† Tumors ½ size of control group

Results of wound irrigation with 5 ml of chemotherapeutic agent 30 minutes after inoculation of a 2 cm wound beneath panniculus carnosus in Strain A mice Evaluation on 14th day

date to the late Nineteenth Century when carbolic acid was employed for this purpose The problem has become better defined since that time with there being good evidence that the free or unestablished tumor cell is much more sensitive to a tumoricidal agent than is the same cell with an established

ılood supply Apparently complete destruction of all neoplastic cells is not ıecessary since the resistance of the host may control tumor growth when the noculum is small [4]

The ideal tumoricidal agent for prevention of tumor implantation in a urgical wound would be one causing maximum destruction of the free neoılastic cell with minimal interference with the normal reparative processes of he host *Although such an agent could be administered either systemically* r locally it is probable that the latter would permit a higher local concentra ion of the agent and fewer over all systemic effects All of the agents studied re cytotoxic and it is likely that the rapidly proliferating neoplastic cell is nore sensitive than the more slowly multiplying normal cells of the host The *harmacologic actions of these drugs would seem to depend upon their ability* o penetrate the cell membrane and then interfere or damage intracellular nzyme systems In no instance however is their mechanism of action well lefined

The action of both Clorpactin® XCB and chloramine T is by way of the iberation of hypochlorous acid and its associated oxidizing potential Both ompounds were fairly effective as cancercidal agents by the three criteria em ıloyed The slower liberation of hypochlorous acid from Clorpactin® XCB nay account for its decreased action for short periods *in vitro* and greater ffectiveness in higher concentrations *in vivo* This mechanism may also be esponsible for the lower toxicity of Clorpactin® XCB *in vivo* as compared o chloramine T Both of these agents have the disadvantage of being inactive n *alkaline media and reacting with all organic matter*

Lugol s solution with similar oxidizing properties was not effective in con entrations well tolerated by the animal This is in keeping with studies dis losing a low cellular toxicity index (against leukocytes and embryonic tissue) lespite a high bactericidal effect

Formalin exerts its cytotoxic action by way of its reactivity with proteins In olutions which are antibacterial it is highly irritating to human tissues From hese investigations it was only moderately effective as a cytotoxic agent in oncentrations tolerated by the animal

Nitrogen mustard in varying concentrations gave the most consistent tu noricidal effect with all methods of appraisal Its cytotoxicity is related to its *ıbility to interfere with nucleic acid metabolism* (primarily cessation of DNA ynthesis) Concentrations of this agent above 2 mg % were highly tumoricidal n both the *in vitro* and *in vivo* studies Concentrations of 1 mg % however vhen used locally produced necrosis of the skin in one mouse and evidence ıf delayed wound healing in others In contrast to the animals receiving hloramine T and Clorpactin® XCB there were no tumor nodules at the site ıf puncture wound of the abdominal wall This finding suggests that the ystemic effect of nitrogen mustard may have been [illegible] *le in controlling umor cells so implanted It is* [illegible] *likely that t*[illegible] be any similar ystemic effect from other agen[illegible]

A study of several ch[illegible] s in an [illegible] l ex[illegible] nentally implanted Ehrlich s [illegible] A str[illegible] that ılkylating agent nitrogen m[illegible] tive [illegible] of [illegible] umor than the other agents [illegible] 2% [illegible] XCB

saline, 5% formalin in saline, 5% chloramine T in saline, and Lugol's solution

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AN ASSAY TECHNIQUE FOR TESTING CANCER THERAPEUTIC AGENTS FOR CLINICAL USE *

JOSEPH A DIPAOLO AND GEORGE E MOORE

Only a few of the large number of compounds tested by microbiologic techniques and against transplanted tumors in animals have been effective in clinical trial against some form of human cancer

Ascites tumor cells *in vitro* have been used to evaluate potential cancer chemotherapeutic agents by determining dehydrogenase inhibition and possibly general cell toxicity [1, 2] Over 125 compounds have been tested using Ehrlich's ascites tumor cells in agar pour plates and in tubes containing agar The compounds which showed positive results are being tested further for cancer chemotherapeutic activity Some known chemotherapeutic agents including actinomycin D, methylbis(B chloroethyl)amine, and a methopterin, responded to these tests

In order to ascertain which chemotherapeutic agents might be most effective against a specific tumor, the ascites agar methods have been modified for testing chemicals directly on suspensions prepared from the patient's tumor

METHOD

Fresh, sterile, human malignant tissue was obtained directly from the operating room The fat and other unwanted tissues were scraped off with a scapel Approximately 1 gm of tumor was minced with scissors, placed in a small beaker, and weighed The mince was transferred to a Tenbrock homogenizer immersed in ice water The weight of the sample was determined by difference The homogenizer was turned by hand and the tumor was homogenized with a 5 0 ml solution of 0 051 M sodium citrate and physiologic saline adjusted to pH of 7 2 and containing 2×10^6 units of penicillin/L

The medium for the agar plates was made as described by Miyamura [3] (peptone, 1 gm, glucose, 0 5 gm, sodium chloride, 0 25 gm, hydrated sodium monohydrogen phosphate, 0 3 gm, agar, 2 gm, and distilled water, 100 ml)

* From the Departments of Experimental Pathology and Surgery Roswell Park Memorial Institute Buffalo Supported in part by a U S P H grant CY 3558

The pH of the medium was adjusted to 7 2 Cellular activity was determined by using a 0 05% methylene blue solution as a redox indicator

Different concentrations of homogenate were used to insure that the reduction of dye would not be too rapid or too slow In order to make a pour plate, 6 ml of medium, 0 5 ml, 1 0 ml, or 1 5 ml of homogenate, and rabbit plasma equal to the amount of homogenate were mixed in a test tube and poured into a petri dish Paper discs containing 100 μg of drug were placed on the agar surface after it had hardened By proper placement of chemotherapeutic agents on the agar, it is possible to test effectiveness of individual compounds as well as any interaction between them Depending upon the time of day at which the tumor was received, the pour plates were refrigerated at 4°C for 10 hours and incubated at 37°C for 8 hours or incubated at 28°C for 18 hours, the former method giving results easier to read Following incubation the discs were washed with distilled water and covered with 10 ml methylene blue After the excess dye was removed, a circular glass plate was placed on the agar, and the petri dishes which had been refrigerated were reincubated for a minimum of 3 hours at 37°C while those which had been incubated only were reincubated for a 5 hour period at 37°C The area of unreduced dye, indicating dehydrogenase inhibition by the test drug, was measured in millimeters

The compounds used in this test contained 1 ml of drug/ml of solution or suspension and the amount placed on a disc was 100 μg The following compounds were used actinomycin D (Merck & Co), N, N', N'' triethylene thiophosphoramide (Lederle), methylbis(B chloroethyl)amine (Merck), nitrogen mustard N oxide (Yoshitomi Pharmaceutical Industries), a methopterin (Lederle), and 6 mercaptopurine (Burroughs Wellcome)

RESULTS

In Table 1 are presented the results obtained with the selected compounds using, in place of the human tumor homogenate, 2 × 10^7 mouse Ehrlich's ascites tumor cells suspended in Tyrode's solution The 6 mercaptopurine, which was reported as having very little dehydrogenase inhibition activity,[4] gave doubtful results which could not be measured Results with another known carcinostatic compound, N, N', N'' triethylene thiophosphoramide, were negative

The results tabulated in Table 2 demonstrate the range of activity of a

Table 1 Inhibition of Mouse Ehrlich Ascites Agar Tests With Known Cancer Chemotherapeutic Compounds

CHEMICAL	ZONE OF INHIBITION IN MM
Actinomycin D	19
A methopterin	18
6 Mercaptopurine	hazy
Methylbis(B chloroethyl)amine	30
Nitrogen mustard N oxide	25
N N', N'' Triethylene thiophosphoramide	0

Table 2 Results of Human Tumor Agar Tests Using Known Cancer Chemotherapeutic Compounds

	Mammary CA cases					Rectal CA cases			Stomach CA cases			Ovary CA cases			Lung CA cases		Parotid CA cases
	1	2	3	1	5	1	2	3	1	2	3	1	2	3	1	2	1
Actinomycin D	15†	20	0	0	12	20	8	15	1›	11	14	0	0	0	0	10	0
A methopterin	0	0	0	0	0	0	0	0	13	0	8	10	0	0	0	8	1_
6 Mercaptopurine	0	20	20	0	10	0	0	0	10	13	0	13	10	13	13	11	0
Methylbis(B chloro ethyl)amine	10	25	15	15	20	1›	0	14	0	0	15	0	18	13	13	12	0
Nitrogen mustard N oxide	0	15	0	13	15	10	0	0	13	0	0	0	11	13	13	0	0
N N N Triethylene thiophosphoramide	15	16	13	0	15	20	10	12	13	14	0	12	20	13	13	15	0

† Figures represent zone of inhibition expressed in mm

variety of cancer chemotherapeutic compounds on human tumors *in vitro*. As might be anticipated, the reaction by a specific tumor type varies even with the same drug.

Several attempts were made to concentrate whole cells from the homogenate by filtering the homogenate through cheesecloth. Unfortunately, it was never possible to obtain more than 5 million cells/ml. Work with mouse ascites has shown that optimal results are obtained with 2×10^7 cells and that interpretable results may be obtained with 10^7 cells in each plate.

Attempts to substitute beef serum or human serum for the rabbit plasma resulted in plates with unreduced dyes in all experimental and control plates. Using 10% human serum in tissue culture medium also failed.

DISCUSSION

Following the demonstration of Warburg and Christian[5] that the plasma of animals with sarcomata has more zymohexase than normal cells and that the tumor cells obtain their energy from glycolysis even in the presence of available oxygen, Straus, Cheronis and Straus[6] were able to show the presence of reducing enzyme systems in neoplasms and living mammalian tissue with triphenyltetrazolium salts. Normal cells would be expected to utilize the more efficient cytochrome system in the presence of adequate oxygen and thus not stain as intensely. This differential reduction of dye by carcinomatous tissue as compared to the rate of reduction of nonmalignant tissue is apparent even when a gauze pack is saturated with tetrazolium salt and applied to ulcerated areas. The presence of malignancy is noted by the rapid change in the dye.

Methylene blue, used in these experiments, or the tetrazolium salts or 2,6-dichlorophenol indophenol that form insoluble colored compounds when reduced, are indicators for dehydrogenases in general and at this time do not differentiate the role of specific reducing enzyme systems.

Since this assay technique depends upon the ability of the drug to inhibit dehydrogenases, drugs which do not inhibit these enzymes would not be expected to produce a zone of inhibition. Furthermore, no *in vitro* test can be expected to reflect the variety of conditions which are encountered clinically. At best this technique may aid in the selection of the most likely chemotherapeutic agent for the first course of therapy.

CONCLUSIONS

1. Sensitivity of tumor tissues to various kinds of carcinolytic drugs varies even with same type of tumor.

2. No relationship between type of tumor and the activity of specific cancer chemotherapeutic compounds is noted.

3. Clinical application of this technique is inferred for the future.

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THE LIMITATIONS AND ADVERSE EFFECTS OF CANCER CHEMOTHERAPY *

TATSUHEI KONDO AND GEORGE L. MOORE

Surgeons are painfully aware of the therapeutic limitations of excisional surgery for most kinds of cancer. Once distant metastases are established, further curative surgical efforts usually are useless. Compounds with anticancer activity are being found weekly but unfortunately none have proved to be effective against the majority of malignant tumors.

The use of several alkylating compounds for the eradication of unestablished tumor cells in conjunction with excisional surgery is being tested clinically at the present time.[7] These and most other anticancer agents have no specific action on cancer cells but are also toxic to normal cells as reflected by severe systemic reactions. Since such reactions may reduce host resistance to tumor growth, instances in which tumor insensitivity to the chemical agent might result in apparent stimulation of tumor growth could be expected. In the treatment of patients with advanced disease by triethylenethiophosphoramide (TSPA), mechlorethamine hydrochloride (HN_2), nitromin and actinomycin D, this phenomenon of an apparent stimulation of local tumor growth and metastases has been observed. This study concerns these clinical observations and confirmatory laboratory experiments.

The treatment of patients with advanced malignancy by TSPA (1.0 mg./kg. i.v. in 5 days) and actinomycin D (75 µg/kg. i.v. in 10 days) has been continued since 1956. Of 91 patients treated with TSPA, 9 exhibited accelerated or explosive growth subsequent to therapy. Similarly increased tumor growth was observed in 1 of 50 patients given actinomycin D.

Unfortunately, statistical evidence from serial clinical measurements of tumor growth and autopsy studies are not of help in substantiating these observations. For example, the number of organs with metastases in breast patients treated with x-ray, hormones, or chemotherapy was greater than in patients without such adjuvant therapy, but the former groups also had a longer survival period during which time metastases could become established.

METHOD

An ascites tumor (Ehrlich) transplanted into Swiss mice was used in the following experiments. Tumor cells suspensions were carefully counted with a hemocytometer and varying numbers of tumor cells injected into a tail vein, the peritoneal cavity, or subcutaneous tissues. Various cancer chemotherapeutic agents were injected into the peritoneal cavity or tail veins. Results of the experiments in which tumor cells were injected into the tail vein were assayed by counting the number of lung metastases determined by macroscopic inspection 50 days after cell injection.

The Effect of Chemotherapeutic Compounds upon the Development of Metastases When Given *Subsequent* to Tumor Cell Injection. In order to demonstrate this phenomenon a combination of a toxic compound and tumor which is relatively insensitive to its action must be used.[6] The senior author has

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* [illegible] m F [illegible]
Ros[illegible] [illegible] the assistance of Gordon Bohn and Joseph Fracasso [illegible]

reported such an experiment in 1956[3] in which Nitromin (the N oxide of mechlorethamine) and Yoshido sarcoma tumor were used

In the following experiment (Table 1) one million Ehrlich ascites cells were injected into the tail vein Two weeks later groups of experimental mice were given 1 to 4 daily doses of 0 5 mg /kg of HN_2 The maximum number of metastases occurred in mice given 2 injections The decreased number of metastases with larger doses probably represents a true chemo therapeutic effect Table 2 illustrates a parallel experiment with actino mycin D

Table 1 Metastases After Posttreatment With HN_2 (1 Million Ehrlich's Cells Injected i v) (HN_2 Injected i v 14 Days Later)

	NUMBER OF METASTASES PER NUMBER OF MICE	NUMBER OF METASTASES PER MOUSE
No HN_2	0/21	0
HN_2 0 5 mg /kg		
× 1 day	4/19	0 2
× 2 days	23/23	1 0
× 3 days	9/18	0 5
× 4 days	5/8	0 5

Table 2 Metastases By Posttreatment With Actinomycin D

	NUMBER OF METASTASES PER NUMBER OF MICE	NUMBER OF METASTASES PER MOUSE
No Actin D	29/36	0 8
Actin D 50γ/kg		
× 1 day	9/29	0 3
× 2 days	86/29	29 7
× 3 days	98/30	32 7
× 4 days	33/28	1 2
× 5 days	14/22	0 6

This study demonstrates host toxicity resulting from an increase in the total dose followed by relatively more effective carcinostasis as the dosage was increased still more

In other experiments carcinostatic levels of the drugs were attained followed by apparent differential toxicity to the host by larger doses as determined by an increased number of metastases

The Effect of *Pretreatment* with Chemotherapeutic Agents upon the Development of Metastases. *As stated previously, in all of these experiments two* elements exist 1) the action of the compound upon the tumor cells, and

2) the effect of the compound upon the host and the local organs which will support tumor growth. By giving the chemotherapeutic agents before the tumor cells are injected, these two factors can be separated. The interval between drug injection and subsequent tumor cell administration must be altered according to the length of the drug's activity. For example, if a long-acting drug such as nitromin is used, there will be a residual effect upon tumor cells injected in the next several days thus prohibiting a clear separation of host and tumor reaction.

Groups of mice were treated with 1 to 5 daily doses of HN_2 (1 mg./kg.). Twenty-four hours after the last injection, two million Ehrlich's ascites cells were injected into the tail vein. The number of lung metastases developing in each group is depicted in Table 3.

Table 3. The Effect of Pretreatment With HN_2 Upon The Establishment of Lung Metastases

	Number of metastases per number of mice	Number of metastases per mouse
No HN_2	8/34	0.2
HN_2 1 mg./kg.		
× 1 day	27/24	1.1
× 2 days	42/22	1.9
× 3 days	34/22	1 6
× 4 days	89/22	4 0

The increase in lung metastases parallels the dosage levels and no apparent effect of the drug on the tumor cells was demonstrated.

The Transplantability of Tumor Cells in Animals Pretreated with Chemotherapeutic Agents. The tumor host relationship of Ehrlich's ascites tumor to our strain of Swiss mice clearly does not simulate an isologous transplantation. Host resistance to the tumor can be demonstrated by tumor regression, transplant failure, and an induction of a nonspecific immunity by suitable injections of dead and live tumor cells

In these experiments tumor transplantation with small numbers of cells was attempted after pretreatment of the recipient animals with a series of 5 daily injections of both short-acting compounds (HN_2, actinomycin D, T.E.M.) and long-acting ones such as nitromin and 6-mercaptopurine. Varying numbers of tumor cells (10 to 100,000) were used in an attempt to determine the critical number of cells for transplantation in intact animals and in those subjected to the carcinostatic agents.

Briefly, there was an increased number of takes and a more rapid growth rate of the tumors in animals pretreated with the short-acting compounds. Several long-acting compounds caused an inhibition of tumor implantation and growth even when the cell injections were delayed 5 days after the last dose of the chemotherapeutic agent.

The Effect of Cortisone upon Tumor Transplantability. Agosia *et al.*[1] first reported the pronounced effect of cortisone upon tumor transplantation.

similarly resulted in accelerated tumor growth presumedly by reduction of host resistance

4 Pretreatment of mice with chemotherapeutic agents increased the transplantability of a minimal number of tumor cells

5 Previous observations that cortisone prepared animals developed a greater number of metastases, an accelerated tumor growth rate, and increased transplantability were confirmed

6 Preliminary experiments indicate that the chemotherapeutic agents may also affect tumor dissemination by affecting cellular adhesiveness

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ETIOLOGY OF INFLAMMATORY RELATIONS IN BREAST CARCINOMA *

JAMES T GRACE, JR AND THOMAS L DAO

Inflammatory reactions associated with breast carcinoma have long been recognized by surgeons Excellent descriptions of the clinical syndrome which we know as inflammatory breast carcinoma appear in early papers [1 2]

While considerable emphasis has been placed on the diagnostic, prognostic, and therapeutic aspects of the disease, relatively little attention has been accorded the mechanisms underlying the inflammatory responses Schumann [3] in 1911 theorized that the cancer cells released a toxic material which caused local irritation Learmonth [4] likewise felt that the cancer cells were irritants However, today it is generally agreed that the heat and redness is not a true inflammation in the sense of a reaction to an irritant or infection The most widely held current concept appears to be that the extensive lymphatic blockage produces the edema and that the associated capillary congestion produces the reddening and heat [5] The round cell infiltration is attributed to lymphatic blockage rather than to a true inflammatory reaction

This concept raises several questions First, if edema due to lymphatic obstruction is the basic mechanism, why don't all cases of breast carcinoma with edema and superficial lymphatic obstruction show inflammatory signs?

* From Surgical Services Roswell Park Memorial Institute, Buffalo Supported in part by USPHS Grant (C1002)

Secondly why do inflammatory responses occur in areas in which there is no edema? Thirdly why don t unrelated conditions causing superficial lymphatic obstruction elsewhere in the body give rise to such responses?

The striking clinical similarity between some of the breast lesions and the local responses observed in some hypersensitivity reactions (such as is observed at the site of penicillin injection in a sensitive subject) prompted us to undertake the studies presented here

Since we felt that the basic mechanisms producing heat and redness in all these lesions were probably similar we did not limit ourselves only to those lesions which met the criteria of true inflammatory carcinoma in the strict clinical sense i e inflammatory response involving more than one third of the breast edema of more than one third of the breast etc[5] Other lesions showing definite areas of redness and heat without apparent cause were also studied The extent of the inflammatory reactions was graded 1 to 4 plus Lesions graded + + + + and + + + met the usual clinical criteria of true inflammatory carcinoma There were 3 of these One plus was reserved for those lesions showing minimal but definite areas of redness and increased heat Two plus represented an intermediate stage

METHOD

A portion of each patient s breast tumor was removed as well as specimens of her normal skin subcutaneous tissue and muscle Saline extracts were prepared from the tumor and the pooled normal tissues The specimens were minced finely with scissors suspended in equal volumes of cold saline and thoroughly homogenized with a mechanical homogenizer The homogenates were centrifuged at 3000 × G for 5 minutes at 4°C The supernatant fluids were filtered through 2 sheets of sterile Whatman #2 filter paper The resulting filtrates constituted the extracts used for testing This material was stored below 4°C until used It was allowed to warm to room temperature prior to injecting

Each patient was tested intradermally with the extract of her normal tissue and the extract of her tumor The reactions were read at 20 minutes The skin test results were graded 1 to 4+ Four plus was a test showing a wheal greater than 1 cm in diameter and surrounding zone of erythema greater than 2 5 cm in diameter One plus was a wheal greater than 0 25 cm with surrounding erythema greater than 1 cm in diameter The skin test injections were carefully performed with very small needles to minimize nonspecific effects due to trauma Some tests were performed blindly with the person actually performing the tests and recording the results unaware of the nature of the extracts

The control group consisted of patients with breast cancer but no evidence of inflammatory reactions

The results of the skin tests are shown in Tables 1 and 2

It is apparent that the patients with inflammatory reactions gave positive results when tested intradermally with extracts of their own tumors Those lesions with the most marked inflammatory reactions gave the strongest positive skin tests All patients gave negative reactions to extracts of their normal tissues Conversely patients with breast carcinoma unassociated with inflammatory reactions gave negative skin tests to both extracts of their tumor and normal tissues

Table 1 Breast Tumors With Inflammatory Reactions

Type	Inflammation	Skin test	Passive transfer
1 Primary	++++	++++	+
2 Primary	++++	++++	+
3 Primary	+++	++	+
4 Primary	++	+	−
5 Recurrent	++	+++	+

Table 2 Breast Tumors Without Inflammatory Reactions

Type	Skin test (tumor extract)
1 Primary	−
2 Primary	±
3 Primary	−
4 Primary	−
5 Primary	−
6 Recurrent	±

In order to establish the specificity of the positive reactions passive transfer studies were carried out in the following manner

Each patient s serum (0 2 cc) was injected intradermally into each forearm of another person Below these injection sites 0 2 cc of control serum obtained from a normal volunteer was injected in like manner All areas were appropriately marked Twenty four hours later skin testing with the tumor extract was done in one of the areas pretreated with the patient s serum control serum and in an untreated area The corresponding areas on the opposite forearm were tested with the extract of the patient s normal tissues Of the 6 skin test sites in each study, only the one pretreated with the patient s serum and challenged with that patient s tumor extract gave a positive reaction A diagram of this study is shown in Table 3

Table 3 Passive Transfer Study

Pretreatment	Challenge: Tumor extract	Challenge: Normal extract
Patients serum	+	−
Control serum	−	−
None	−	−

+ Positive reaction
− Negative reaction

DISCUSSION

These studies demonstrated that these patients were sensitive to some constituents of their own tumors The patients negative reactions to their normal tissue extracts suggested a tumor specific sensitivity This specificity was established by the passive transfer studies These studies eliminated the possibility that histamine like substances in the tumors accounted for the positive skin tests with the tumor extracts since such agents would have given a positive result when injected into the skin of other persons The humoral nature of the antibodies involved was demonstrated by the immediate type responses and by transferral of the sensitivity with the patients sera

It is postulated that the underlying mechanism of many of the inflammatory reactions associated with breast carcinoma is the patients sensitivity either to their own tumor or to products of their tumor The significance of this sensitivity in terms of the patients responses to their disease needs further study since these tumors carry notoriously poor prognoses

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AN ESTRADIOL SENSITIVE TRANSHYDROGENASE IN NORMAL AND MALIGNANT BREAST TISSUE *

FALLS B HERSHEY

Cancer of the breast is functionally classified into two groups The growth of the first is independent of known hormonal influences The second type has been termed estrogen dependent and regresses after removal of the adrenals or ovaries This paper describes estrogen stimulation of an enzyme a transhydrogenase which is a possible mechanism of the hormone s effects *in vivo* This *in vitro* test may serve to distinguish the autonomous and the estrogen dependent cancers so that the disease can be treated more rationally

The enzyme catalyses the transfer of hydrogen between two pyridine nucleotide coenzymes †



$$TPNH + DPN \rightleftharpoons DPNH + TPN$$ (Line B Fig 1)

† Abbreviations used in this paper include DPN and DPNH for oxidized and reduced diphosphopyridine nucleotide respectively TPN and TPNH for oxidized and reduced triphosphopyridine nucleotide ICDH for the enzyme isocitric dehydrogenase LDH for the enzyme lactic dehydrogenase and TH for the enzyme pyridine nucleotide transhydrogenase

* From the Department of Surgery Washington University School of Medicine and the U S Veterans Hospital St Louis Supported in part by the John M Matthews Memorial grant from the American Cancer Society the U S Public Health Service (RG 4192) and the Harry Freund Memorial Foundation

This paper reports its occurrences in normal and malignant breast tissues In some cases the reaction is markedly accelerated by minute quantities of estradiol

Another reaction in placenta[1] and endometrium[2] and breast[3] has been observed to be stimulated by estradiol

$$\text{Isocitrate} + \text{DPN} \rightleftarrows \alpha\ \text{ketoglutarate} + CO_2 + \text{DPNH}_{+H^+} \quad \text{(Line C Fig 1)}$$

This reaction required DPN and was interpreted as evidence for a different ICDH enzyme We have confirmed these observations in placenta and breast tissue Recently Talalay[4] and Villee[5] presented indirect evidence that this apparent stimulation of the placental DPN linked ICDH was actually due to coupling of the ordinary TPN specific ICDH reaction (Reaction a Fig 1) with a transhydrogenase reaction (Reaction b Fig 1) We have observed the same phenomenum in breast tissue and have measured the over all coupled reaction and in addition have measured the two components of the coupled reaction separately

METHOD

Preparation of Homogenates Fresh samples of cancer and normal breast tissue were obtained and 10% homogenates were prepared in 0 25 M sucrose with glass homogenizers for 3 minutes Reagents and vessels were iced Unused tissue was frozen quickly Homogenates were centrifuged and the fatty layer and sediment were discarded

Fluorometric Measurements of Enzyme Reactions DPN dependent ICDH (Reaction c) was measured by fluorometric methods For reasons discussed later this is called the coupled transhydrogenase system The enzyme activity is expressed as the rate of appearance of reduced DPNH Readings of the fluorescence were taken every few minutes after the addition of the tissue and the tubes were incubated at 38° between readings Readings were continued for 1 to 1½ hours Various materials were added to the reaction mixture and their effects noted Some fluorescence results from TPNH formation also but causes no confusion The TPNH remains but the DPNH immediately disappears when LDH and pyruvate are added at the completion of the reaction They convert the DPNH to the nonfluorescent DPN The fluorescence of the reaction mixture is compared with readings for standard solutions of DPNH Reoxidation of the DPNH by competing reactions was examined by addition of homogenate to known amounts of DPNH Direct measurements of the transhydrogenase were also made with DPNH and TPN as substrates TPN dependent (Reaction a) ICDH was measured with our fluorometric method[6] Composition of the reaction mixtures is shown in Table 1

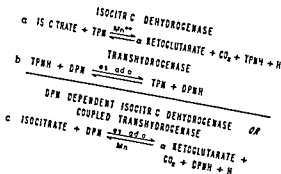


Fig 1 Estrogen sensitive enzyme reactions in breast

The sensitivity of these fluorometric methods is 1000 times the sensitivity of spectrophotometric measurement of DPNH or TPNH and permitted accurate measurements with only 3 mg of tissue in 1 ml reaction volume

Table 1 Composition of Reaction Mixtures

	ICDH (TPN LINKED)	COUPLED TRANSHYDROGENASE METHOD	DIRECT TRANSHYDROGENASE METHOD
Tris pH 7 38	0 15 M/L	0 15 M/L	0 15 M/L
DPN	—	0 36 mM/L	None
DPNH	—	—	0 02 mM/L
Mn CL_2	0 55 mM/L	0 55 mM/L	—
d Sod Isocitrate	6 0 mM/L	6 0 mM/L	—
Estradiol 17β	1μg/ml	1μg/ml	1μg/ml
Tissue	3 5 mg	3 5 mg	1 2 mg /0 05 ml
TPN	1 mM/L	0 004 mM/L added after baseline	0.2 mM/L
Reaction volume	1 0 ml	1 0 ml	0 05 ml
Incubation time	30 90 minutes	30 90 minutes	30 minutes
Incubation temp	38° C	38° C	38° C

Other Determinations. Protein content of the homogenates was measured by the method of Lowry, using a calibration curve made with human serum[7] Desoxyribonucleic acid was measured by an unpublished fluorometric method

Oxidized and reduced DPN and TPN, lactic dehydrogenase (LDH) isocitric acid and isocitric dehydrogenase (ICDH) were obtained from Sigma Chemical Company, estradiol 17β from the Upjohn Company, disodium versenate from Fisher Scientific Company

RESULTS

Properties of the Enzyme. The enzymes were all present in the supernatant fraction of the homogenates of normal breast and cancer centrifuged for 10 minutes at 3000 × g Frozen homogenates maintained their activity for at least 7 days at −20°C Frozen tissue maintained activity for several weeks at −85°C There is an apparent loss of TH activity of cancer tissue when stored for longer times However, it is difficult to compare the enzymic activities of homogenates of different samples of cancer containing tissue because the samples differ in their proportions of cancer cells, fat and stroma

Repeated determinations of the same homogenate agree ±5% The DPNH produced was proportional to the amount of tissue added The rates of formation of DPNH were calculated from the linear phase of the reaction which occurs about 5 minutes after the addition of very small amounts of TPN Figure 2 shows a typical experiment The fluorescence was identified as DPNH because of its disappearance when LDH and pyruvate were added at the completion of the reaction They convert the DPHN to nonfluorescent DPN Some homogenates simultaneously reoxidized part of the DPNH produced Fortunately, this competing reaction occurs infrequently and at a relatively slow rate and is unaffected by estrogen Measures to eliminate

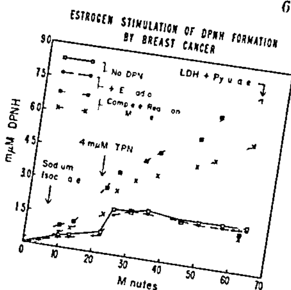


Fig 2 Estrogen stimulation of DPNH formation by DPN dependent isocitric dehydrogenase or coupled transhydrogenase of breast cancer Composition of reaction mixture is given in Table 1

this are being devised DPNH formation from other reactions with endogenous substrates is not excluded but appears to be unimportant

Estrogen stimulation could be expressed various ways first by comparing the slopes of these two lines and also by comparing the differences in DPNH revealed at the end of the experiment after the addition of LDH or by comparing only the differences in total fluorescence at the end of the experiment The precise magnitude of the estrogen stimulation remains uncertain since there is undoubtedly some endogenous estrogen and since there is probably some DPNH formed from other concurrent reactions which are not estrogen dependent At the present no significance is attributed to estrogen stimulation of less than 15% Striking stimulation may occur The rate of formation of DPNH may be quadrupled by the addition of estrogen When stimulation occurs in the cancer tissue it has also been found in the accompanying normal tissue in all cases tested thus far Estrogen stimulation may be observed both with the most active and with the least active homogenates

The analysis of the cancer tissue from 9 individuals is recorded in Table 2 In most instances nonmalignant tissue was also examined The activities of the ICDH and of the coupled transhydrogenase showed considerable variation The activity of the ICDH ranged from 1240 to 9710 mM/per kg protein/hour This is roughly 25 to 100 times the activity of the coupled transhydrogenase in the same specimens The coupled TH in these 9 cases ranged from 0 3 to 73 mM/kg protein/hour Variances of ICDH and TH were not parallel

Desoxyribonucleic acid content of the homogenates has also been measured The activity of ICDH and of TH also showed a wide range when calculated per milligram DNA The activity of the cancer homogenates sometimes exceeded that of the nonmalignant breast tissue The coupled transhydrogenase was also tested on a sample of metastatic cancer of the prostate It showed a rate of 1 3 mM/kg/hr with an estrogen stimulation of 30%

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The sensitivity of these fluorometric methods is 1000 times the sensitivity of spectrophotometric measurement of DPNH or TPNH and permitted accurate measurements with only 3 mg of tissue in 1 ml reaction volume

Table 1 Composition of Reaction Mixtures

	ICDH (TPN LINKED)	COUPLED TRANSHYDROGENASE METHOD	DIRECT TRANSHYDROGENASE METHOD
Tris pH 7.38	0.15 M/L	0.15 M/L	0.15 M/L
DPN	—	0.36 mM/L	None
DPNH	—	—	0.09 mM/L
Mn CL_2	0.55 mM/L	0.55 mM/L	—
d Sod Isocitrate	6.0 mM/L	6.0 mM/L	—
Estradiol 17β	1μg/ml	1μg/ml	1μg/ml
Tissue	3.5 mg	3.5 mg	1.2 mg/0.05 ml
TPN	1 mM/L	0.004 mM/L added after baseline	0.2 mM/L
Reaction volume	1.0 ml	1.0 ml	0.05 ml
Incubation time	30 90 minutes	30 90 minutes	30 minutes
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Other Determinations Protein content of the homogenates was measured by the method of Lowry using a calibration curve made with human serum Desoxyribonucleic acid was measured by an unpublished fluorometric method

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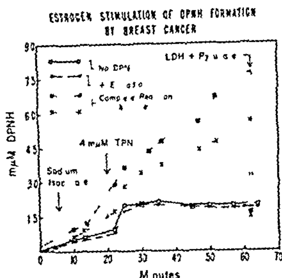


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Table 2 Enzyme Analysis of Cancerous and Noncancerous Breast Tissue

		ICDH	COUPLED TRANSHYDROGENASE			
			NO ESTRADIOL		—ESTRADIOL	% STIMULATION
CASE	AGE	mM PER KG PROTEIN PER HOUR	mM PER KG TISSUE PER HOUR †	mM PER KG PROTEIN PER HOUR	mM PER KG PROTEIN PER HOUR	
H P	57	CA 4660	229	6 26	11 1	78%
I S	60	CA 2880	168	16 08	16 0	
		NON 2940	10	3	3	None
I E	64	CA 9250	224	6 1	6 1	
		NON 6750	10	3	3	None
F R	42	CA 3500	77	2 85	2 8	None
M McC	46	CA 2670	1051	40 6	40 6	None
		NON 2280	695	63 7	73	15%
E S	49	CA —	230	1 03	1 2	13%
M J	49	CA 9710	455	—	† 0 6	28%
		NON 1240	235	6 84	13 5	100%
R S	46	CA —	778	31 2	38 8	25%
H F	54	CA —	6350	—	† 7 7	18%

Columns 1 3 and 4—activity is expressed as millimoles DPNH produced per kg protein per hour

† For these two homogenates activity is expressed millimoles DPNH produced per kg wet weight of tissue per hour

All rates were measured during the linear portion of the curves 5 minutes after the addition of catalytic amounts of TPN Estrogen stimulation was calculated by comparison of these rates

and TPN as substrates (Table 1) and also in the reverse direction The stimulation of this reaction by estrogen is evident only with low concentrations of the substrates, particularly DPNH More work is needed to clarify the nature of the estrogenic stimulation of these reactions

Additional evidence for the coupled nature of the reaction might be obtained by fractionation of the two components however sufficient tissue has not been available for this Addition of purified ICDH from pig heart has not increased the activity of the coupled reaction or restored it when it has been lost by storage

DISCUSSION

Much more basic biochemical work is necessary in order to define the conditions for and the significance of the estrogenic stimulation of enzyme activity in these tissues

Mammary cancer from 2 patients in whom testosterone and stilbestrol treatment had failed, showed no estrogen stimulation of transhydrogenase *in vitro* Mammary cancer from a premenopausal patient who subsequently had an oophorectomy showed 28% stimulation and her cancer has regressed considerably Another patient, who subsequently had an hypophysectomy, *showed 21% stimulation of coupled TH It is still too early to evaluate the* result of this operation More analyses must be done on patients who will need hormone treatment or ablative operations

When one considers the various methodological factors which may influence the coupled reaction it appears that the simpler system, namely the direct method for measurement of transhydrogenase, will be more satisfactory than the coupled system. Work is in progress to apply this method to more samples of breast cancer. However, better answers to some of the problems concerned, and the elucidation of the mechanisms of hormone dependence of tumors may require the microanalysis of groups of pure cancer cells, dissected from surrounding stroma and fat. Identification of groups of cancer cells in freeze-dried and unstained sections of breast cancer is not difficult, particularly after comparison with alternate sections which have been cleared and stained. Micromethods for some enzymes have been highly satisfactory in microgram samples of various layers of skin.[6] Further refinement of our present methods may permit the analysis of 0.02 mg. of cancer cells from freeze-dried sections of breast.

CONCLUSIONS

1. Analysis for an estrogen sensitive pyridine nucleotide transhydrogenase, and for isocitric dehydrogenase has been conducted in 13 samples of cancerous and noncancerous breast tissue from 9 patients.
2. The transhydrogenase in 5 samples from 4 patients showed 18 to 98% increase in activity when estradiol was added, in the amount of 1 μg/ml.
3. Estrogen sensitivity of the "DPN dependent isocitric dehydrogenase" of breast tissue is most likely due to the coupled transhydrogenase.
4. The relationship between estrogen sensitivity of cancer tissue *in vitro*, and the response to hormone treatment and ablative operations is under investigation.

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ABLATION OF ADRENAL ESTROGENS IN THE TREATMENT OF FAR ADVANCED MAMMARY CANCER *

GEORGE E. BLOCK, JACK D. MCCARTHY, AND A. BURGESS VIAL

The epoch making work of Huggins [1] brought the realization that objective remissions from far advanced breast cancer will occur in approximately 40% of women undergoing bilateral adrenalectomy. The disappointing failures of adrenalectomy have led us to investigate the urinary excretions of estrogens in these patients in the hope that we might better understand the mecha-

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nisms whereby palliation is achieved A secondary aim has been the establishment of criteria for the selection of candidates for adrenalectomy

METHOD

Twenty seven female patients suffering from far advanced mammary cancer were subjected to simultaneous bilateral adrenalectomy after the variable of ovarian function had been eliminated Of these 18 had previously undergone bilateral oophorectomy 7 had been subjected to x ray castration by our standard castration technique [2] and 2 of the patients were 18 or more years post menopausal In this latter group estrogen excretions indicated that what estrogen production remained was not ovarian in origin and was perhaps solely from the adrenal gland [3]

In this series estrogen excretions were determined prior to operation and again postoperatively after the patient was stabilized on a *maintenance dose* of steroid replacement Patients were not selected for operation on a basis of estrogen excretion but because they suffered from far advanced breast cancer and their ovarian status had been ascertained

Remissions were judged only by the generally accepted objective indices [4] and subjective phenomena were not accepted Our criteria for objective re*missions were in general that the majority of lesions had regressed* in some way that we could measure by physical means and that no new lesions had appeared A minimum time period was arbitrarily chosen to define remissions It was felt that if remissions were to be clinically acceptable they must persist for a period of at least 60 days

In the early portion of the study estrogen excretion was measured by a method of bioassay after Smith and Smith [5] A 24 hour urine specimen was extracted and the estrogenic substances determined by the change in the vaginal mucosa of 2 standardized rats Consequently the minimal positive response was less than 2 rat units—trace We have found that a completely negative response in bioassay determination is less than 1 μg by chromatographic methods Recently we have relied upon a paper chromatographic method to determine total estrogen excretion as well as the fractions of estrone estradiol and estriol The method is essentially a modification of the technique of Zaffaroni in which the estrogens are extracted and converted to their hydrazone derivatives after which they are measured by chromatographic methods in comparison with known pure standards [6]

RESULTS

Table 1 shows preoperative and postoperative estrogen excretions A fall in the estrogen excretion was noted following adrenalectomy In 3 cases however preoperative determination by the bioassay method was below the level of sensitivity and therefore no fall could be demonstrated

Preoperative Total Estrogen Excretion (Fig 1) None of the 11 patients who had a preoperative total estrogen excretion of 1 5 μg per day or less experienced a remission Four of the 16 patients whose preoperative total estrogen excretion was between 0 and 2 μg/day obtained remissions (25%) It should be noted that the 11 patients whose estrogen excretion was below 1 5 μg includes patients whose absolute value by bioassay showed no trace of estrogen and therefore could only be said to have less than 2 μg/day of estrogen excretion (probably less than one) Of the 5 patients whose estrogen

Table 1 Pre and Postoperative Estrogen Excretions

PATIENTS SUBJECTED TO BILATERAL ADRENALECTOMY

PATIENT	AGE	OVARIAN STATUS	DATE	ESTROGEN EXCRETION PRE OPERATIVE Total	E1	E2	E3 *	ESTROGEN EXCRETION POST OPERATIVE Total	E1	E2	E3 *	REMISSION	LENGTH OF REMISSION
G B	53	X Ray Cast	2/11/58	3 2	2 0	0 3	0 4	0 47	0 3			YES	12 months
M D	49	Post Cast	3/ 6/58	5 5	1 9	0 8	2 8	0 91	0 24	0 4	0 27	NO	
A E	49	Post Cast	2/ 7/58	4 55	5 2	0 32	0 75	0 56	0 2	0 28		YES	3 months
L F	61	20 yr PM	3/25/58	1 9	1 6	0 3		0 26	0 26			YES	3 months
B F	40	Post Cast	1/ 7/58	0 78	0 56		0 22	0 77		0 51	0 26	NO	
A G	47	X Ray Cast	2/25/58	3 8	2 8	0 7	0 3	1 0	1 0			YES	5 months
S J	41	X Ray Cast	3/24/58	1 6	1 6			0 3		0 5		YES	4 months
R L	67	Post Cast	4/19/56	1 8	1 8			0 48			0 48	YES	3 months
V L	61	X Ray Cast	2/ 3/58	1 6	0 67	0 65	0 28	0 22	0 17	0 05		YES	5 months
A P	43	Post Cast	2/11/57	6 0				<2	0 33			YES	17 months
J R	45	Post Cast	4/25/58	1 67	1 5		0 17	1 17			1 17	NO	
E S	46	X Ray Cast	5/ 2/58	0 90	0 62	0 24	0 04	0 33	0 13			NO	
[illegible]	[illegible]	[illegible] PM	[illegible]	5 0	5 0							YES	2 months
R D	58	X Ray Cast	10/15/56	<2				<2				NO	
C D	55	Post Cast	10/14/56	5 0				<2				YES	9 months
M G	52	Post Cast	12/4/56	1 2				1				NO	
Y C	39	Pos Cast	10/23/57	0 6	0 2	0 3	0 3	0				NO	
L H	60	Post Cast	3/11/58	6 6	2 2	1 4	3 0	0 44	0 26	0 18		YES	2 months
S M	46	Pos Cast	12/2/57	4 13	1 2	2 9	0 2	0 1	0 1			NO	
I R	38	Post Cast	11/8/56	<2				<2				NO	
V S	37	Post Cast	6/26/57	4 6	2 6		?	<2				NO	
M S	38	Post Cast	2/ 6/58	<2				expired				NO	
A S	60	Post Cast	11/19/57	10 2	4 1	4 1	2 5	<2				YES	2 months
C S	37	X Ray Cast	10/22/57	<2	0 8			<2				NO	
D Y	51	Post Cast	10/30/57	5 6	2 6			1 9	1 2	0 64	0 04	NO	

* E1-Estrone E2 Estradiol, E3 Estriol

** remission continue at time of writing

< less than two micrograms or "rat units"

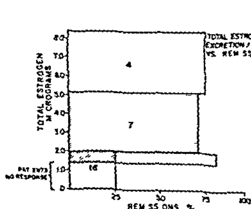


Fig 1 Comparison of preoperative total estrogen excretion with remission rate following adrenalectomy

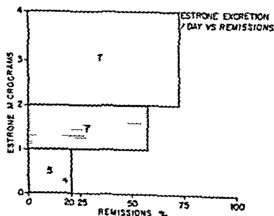


Fig 2 Comparison of preoperative estrone excretion with remission rate following adrenalectomy

excretion fell between 1.5 and 2.0 μg./day, 4 or 80% were benefitted by the procedure. Of the 7 patients whose estrogen excretion was between 2 0 and 5 0 μg./day, the remission rate was 71.4%. Patients who had an excretion of over 5 0 μg. had a remission rate of 75% (3 of 4 cases).

Preoperative Estrone Excretion (Fig. 2). Nineteen patients had a preoperative estrone determination by chromatographic means. The lowest excretion rate associated with remission was 0 67 μg./day. The 5 patients whose excretion was between 0 and 1.0 μg. had a remission rate of 20%; those whose estrone excretion fell between 1.0 and 2.0 μg. (7 patients) had a remission rate of 57.1%. Of the 7 patients whose estrone excretion was in excess of 2.0 μg./day 71.4% experienced remissions.

Preoperative Estriol Excretion (Fig. 3). Both success and failure were encountered at relatively low levels of estriol excretion. In all, 12 patients had preoperative estriol determinations. Thirty-three per cent of the 6 patients having estriol excretions between 0 and 0.3 μg underwent postoperative remissions. The group of patients whose excretion fell between 0 5 and 3 0 μg / day underwent remissions in 50% of the cases. Three of the 9 patients (33%) who excreted between 0 and 1 0 μg. of estriol per day received remissions from the tumor. Of the 3 patients whose estriol excretion was between 1 0 and 3 0 μg /day, 2 underwent remission (66%).

Preoperative Estradiol Excretion (Fig. 4). Ten patients had preoperative determinations of estradiol. Again, success and failure was encountered at relatively low levels of estradiol excretion. Between 0 and 0.5 μg. of estradiol excretion was associated with remissions in 2 of the 4 patients (50%) Seventy-five per cent of the 4 patients whose estradiol excretion was between 0.1 and 1 0 μg improved following adrenalectomy. The group of 8 patients whose excretion was between 0 and 1.0 μg experienced a remission rate of 62.5%. The 2 patients who excreted more than 1 μg. of estradiol in 24 hours experienced postoperative remissions.

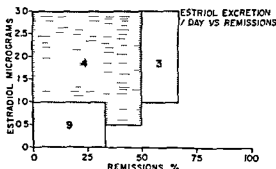


Fig. 3 Comparison of preoperative estriol excretion with remission rate following adrenalectomy

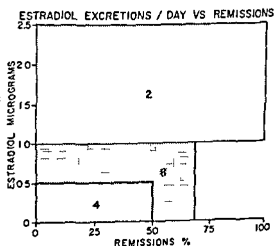


Fig. 4 Comparison of preoperative estradiol excretion with remission rate following adrenalectomy.

DISCUSSION

We have considered a problem in which minute amounts of estrogen are measured. The possibility of error is great. Because of this and because of the very nature of the disease in question, a much larger series than we have presented must be collected if valid conclusions are to be made.

Since we have altered only the endocrine environment of the host by removing the adrenals, after eliminating the possibility of ovarian function, we have assumed that any difference in pre and postoperative determinations of estrogen excretion reflected a decrease in adrenal estrogens. Engel[3] has stated that estradiol is primarily a gonadal estrogen, although small amounts of this most active steroid are manufactured by the adrenals. Estrone and estriol, on the other hand, are primarily adrenal in origin.

There appears to be a critical level of between 1.5 and 2.0 μg. of total estrogen excretion which must be present in order for adrenalectomy to benefit the host. Below this level no patient received any benefit from the procedure. About this level, regardless of the magnitude of excretion, about three-quarters of the patients underwent remissions. This would indicate that minute amounts of total estrogens, as reflected by the urinary excretion, are not sufficient to maintain an endocrine-sensitive tumor and that attempts at palliation by ablative therapy in this sterile media are hopeless.

Estrone and estriol represent the profile of adrenal estrogen activity.[3] The magnitude of their excretion bears almost a linear relationship with the success of adrenal ablation. Although only very small amounts of each were present in some of the successful cases, there is an indication that the three measured fractions taken together as a total combine to effect a favorable tumor environment. Increasing amounts of estrone and/or estriol excretion presages a successful outcome for palliation in a majority of the patients.

Estradiol determinations again had a linear relationship to the success of adrenalectomy. Perhaps this again signifies the minute amount of estrogenic activity of the adrenal. It is reasonable to assume that increasing amounts of estradiol in a castrate female would indicate adrenal activity and, therefore, a greater probability of remission with adrenal ablation.

We did not find the theory of "impeded estrogens" demonstrated in this study.[7] Huggins' experience of estriol acting to retard growth when present in large quantities was not duplicated. Indeed, the converse was true—the greater the estriol excretion, the better the chance for remission following adrenalectomy.

This report deals with only one facet of tumor growth—that of the internal endocrine environment of the host. The inherent biologic properties of the invader have not been directly measured other than by chance association in a media high or low in estrogen content. It is reasonable to assume that tumors flourishing in a climate of high estrogen concentration will suffer from a reduction of this concentration and that tumors thriving in a media almost devoid of estrone will not be greatly affected by further decreasing the hormone.

CONCLUSIONS

1. Bilateral adrenalectomy in the absence of ovarian function lowers the urinary excretion of total estrogens as well as the fractions of estrone, estradiol and estriol.

excretion fell between 1.5 and 2 0 μg./day, 4 or 80% were benefitted by the procedure. Of the 7 patients whose estrogen excretion was between 2.0 and 5.0 μg./day, the remission rate was 71.4%. Patients who had an excretion of over 5.0 μg. had a remission rate of 75% (3 of 4 cases)

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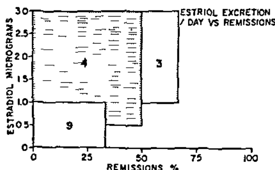


Fig 3 Comparison of preoperative estriol excretion with remission rate following adrenalectomy

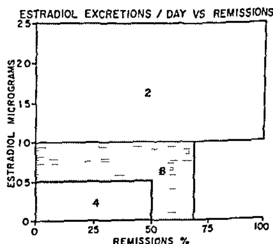


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Since we have altered only the endocrine environment of the host by removing the adrenals after eliminating the possibility of ovarian function, we have assumed that any difference in pre and postoperative determinations of estrogen excretion reflected a decrease in adrenal estrogens. Engel[3] has stated that estradiol is primarily a gonadal estrogen, although small amounts of this most active steroid are manufactured by the adrenals. Estrone and estriol, on the other hand, are primarily adrenal in origin.

There appears to be a critical level of between 1.5 and 2.0 μg of total estrogen excretion which must be present in order for adrenalectomy to benefit the host. Below this level no patient received any benefit from the procedure. Above this level, regardless of the magnitude of excretion, about three quarters of the patients underwent remissions. This would indicate that minute amounts of total estrogens, as reflected by the urinary excretion, are not sufficient to maintain an endocrine sensitive tumor and that attempts at palliation by ablative therapy in this sterile media are hopeless.

Estrone and estriol represent the profile of adrenal estrogen activity.[3] The magnitude of their excretion bears almost a linear relationship with the success of adrenal ablation. Although only very small amounts of each were present in some of the successful cases, there is an indication that the three measured fractions taken together as a total combine to effect a favorable tumor environment. Increasing amounts of estrone and/or estriol excretion presages a successful outcome for palliation in a majority of the patients.

Estradiol determinations again had a linear relationship to the success of adrenalectomy. Perhaps this again signifies the minute amount of estrogenic activity of the adrenal. It is reasonable to assume that increasing amounts of estradiol in a castrate female would indicate adrenal activity and, therefore, a greater probability of remission with adrenal ablation.

We did not find the theory of "impeded estrogens" demonstrated in this study.[7] Huggins' experience of estriol acting to retard growth when present in large quantities was not duplicated. Indeed, the converse was true—the greater the estriol excretion, the better the chance for remission following adrenalectomy.

This report deals with only one facet of tumor growth—that of the internal endocrine environment of the host. The inherent biologic properties of the invader have not been directly measured other than by chance association in a media high or low in estrogen content. It is reasonable to assume that tumors flourishing in a climate of high estrogen concentration will suffer from a reduction of this concentration and that tumors thriving in a media almost devoid of estrone will not be greatly affected by further decreasing the hormone.

CONCLUSIONS

1. Bilateral adrenalectomy in the absence of ovarian function lowers the urinary excretion of total estrogens as well as the fractions of estrone, estradiol and estriol.

2 No patient underwent remission following adrenalectomy if the total estrogen excretion prior to operation was less than 1 5 μg Above this seemingly critical level about three quarters of the patients studied underwent remission

3 Excretions of estrone estriol and estradiol had a direct almost linear relationship with the successful outcome of adrenalectomy

4 The phenomena of impeded estrogens (i e the estriol fraction) was not demonstrated in this study

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STUDIES ON THE MECHANISM OF ACTION OF ADRENAL STEROIDS USING ISOLATED CELL SYSTEMS *

ALLAN D BASS JAMES A SETLIFF AND CATHERINE C SNELL

Although the general responses of the body to adrenal steroid administration are well known and precisely described the mechanism of action of these agents is still incompletely elucidated There have been a few isolated reports during the last 3 or 4 years indicating that hydrocortisone and steroids of similar structure have actions which can be demonstrated in tissue culture[1] in isolated cell systems[2, 3] and on isolated strips of smooth muscle[4, 5] Because corticoids have such a wide variety of metabolic actions it seemed reasonable to assume that the mechanism of action could best be explained by assuming that an important locus of action is at or in the cell membrane For example modification of the permeability of these membranes to various substrates or metabolites could explain many of the steroid s actions We therefore undertook our present study on the hypothesis that some if not all of the many local and systemic changes induced might be due to modification in the function of cellular membranes

METHODS AND RESULTS

Ehrlich s ascites tumor cells were suspended in their own ascitic fluid heparinized to prevent clotting incubated with cortisone acetate suspension

* From the Department of Pharmacology Vanderbilt University School of Medicine Nashville Supported by research grants from the National Cancer Institute of the National Institutes of Health U S Public Health Service (Grant No C 2070) and an Institutional Cancer Research Grant from the Anna Fuller Foundation

at 37°C and suspended in 85% saline at room temperature Changes in cell size were determined by hematocrit measurements after centrifuging to constant volume Cell water was determined by centrifuging an aliquot of the cell suspension weighing and drying to constant weight The results are presented in Table 1 The form of the suspended steroid is important This may be due in part to crystal size but is also in part related to the nature of the crystal surface Fine crystals referred to in the table were prepared by dissolving the compound in acetone and precipitating by rapidly adding a large volume of saline centrifuging and washing with saline to remove traces of acetone The effective concentrations of the steroids are less than those indicated in the table as a significant amount of the agent remains in suspension

Table 1 Effect of Certain Steroids on Ehrlich s Ascites Cells

	CONCENTRATION MG/ML	CELL WATER % CONTROL	PACKED CELL VOLUME % CONTROL
CONTROL		100	100
CORTISONE ACETATE			
Cortone acetate —Merck	5		125
Cortone acetate"—Merck	3 1	118.6	
Coarse crystals	3 1	100	
Fine crystals	3 1	134 1	
DESOXYCORTICOSTERONE ACETATE			
Coarse crystals	3 1	105 6	
Fine crystals	3 1	131.8	
Fine crystals	250	118 4	
HYDROCORTISONE			
Fine crystals	3 1	125 1	
9α—FLUOROHYDROCORTISONE ACETATE			
Fine crystals	3 1	134.5	

Desoxycorticosterone acetate (DOCA) at a concentration of 250 μg/ml increased the cell water to 115% of that of the controls That these are not

… to low lipid solubility causing less reaction with the cell membrane

In view of the fact that these steroids have been shown by Pletscher[2] to affect glucose transport through erythrocyte membranes and by Wilson[3] to affect leucocyte cation transport studies were initiated to determine shifts in cations caused in this system

An aliquot of the tumor cells suspended in their own ascitic fluid was added to each of 2 flasks containing 10 times the tumor volume of ice cold saline One flask contained cortisone acetate suspension at a level of 1 mg /ml The control flask contained an equivalent amount of the suspending medium of the drug Samples were removed periodically from both flasks The cells were separated by centrifuging and washing with 10% sucrose Sodium and

potassium were determined on lysates of the cells by means of a flame photometer The results are shown in Table 2 The rate of loss of potassium is increased and the rate of sodium uptake by the cells is reduced The movement of these ions is not entirely interdependent as potassium is lost more rapidly than sodium diffuses into the cell These ion shifts are independent of cell respiration as they occur at temperatures which arrest the metabolic functions of the cells The steroids therefore are modifying the passive movement of these ions

Table 2 The Effect of Cortisone Acetate on Intracellular Cations

TIME HRS	POTASSIUM		SODIUM	
	CONTROL	TREATED †	CONTROL	TREATED †
0	100	86.7 ††	100	10[illegible].0
½	82.3	68.7	89.5	100.3
1	78.4	69.9	99.4	116.8
2	52.1	42.9	110.[illegible]	112.8
3	47.4	41.8	113.8	109.[illegible]
4	43.0	38.3	127.5	119.[illegible]
5	41.9	33.5	142.2	120.3

† Treated cells were suspended with cortisone acetate suspension at a level of 1 mg/ml
†† The reduction in K at time 0 represents the change which occurs during the 10 minutes required for separation of the cells from the substrate
Figures given represent deviations from the control which is given as *100%* at *time 0* These figures are the averages of *5* separate experiments

Kline[1] reported that He La cells grown in the presence of hydrocortisone showed fewer nuclei per flask and larger cells than the controls This we have confirmed and in addition have observed in 11 day cultures from flasks containing 50 μg of hydrocortisone sodium succinate in the culture media a 48% increase in protein nitrogen per cell an increase of 25% in ribonucleic acid (PNA) per flask an increase of 60% in PNA per cell and an increase of 12.7% in deoxyribonucleic acid per cell Furthermore hydrocortisone causes a decrease in the rate of mitosis of about 50% as determined by the standard Colchicine technique These observations clearly indicate a significant *in vitro* action of the steroid The site of action however has not yet been determined

It is generally agreed that the action of cholinergic drugs on smooth muscle is accompanied by changes in the permeability of cells to electrolytes It was of interest therefore to know whether cortisone and similar steroids would modify the action of pilocarpine and acetylcholine The isolated guinea pig ileum was selected for our test system Cortisone acetate has been investigated most thoroughly although the other steroids tested appeared to have a similar action

The action of cortisone acetate itself is best shown using ileum aged 3 days in Tyrode solution at 2 to 5°C Administration of the drug causes a reduction of both tone and amplitude of spontaneous contraction An ileum which has been contracted by acetylcholine or pilocarpine is promptly relaxed by cortisone acetate and prior administration of the steroid blocks action of pilocar

Fig 1 The effect of cortisone acetate suspension on the response of isolated guinea pig ileum to pilocarpine and histamine (A) pilocarpine—0.57 µg/ml (B) histamine diphosphate—0.016 µg/ml (C) cortisone acetate—7.1 µg/ml The upper figures indicate the normal response to pilocarpine and histamine respectively The lower figure illustrates the ability of cortisone acetate to prevent a significant response to pilocarpine while having little effect on the histamine response

pine and acetylcholine (Fig 1) At the dose levels used there is little or no effect on histamine induced contractions In this respect cortisone acetate seems to have an atropine like action

DISCUSSION

As appropriate *in vitro* systems are devised for testing these steroids it becomes more apparent that they are capable of exerting a direct action on cells and tissues free from the concomitant effects of other hormones Although the precise mechanism of their mode of action is as yet unclear, increasing evidence points toward an ability of these compounds to alter the permeability of the cell This has been demonstrated by the increased swelling of tumor cells and alterations of sodium and potassium content of tumor cells in con t ... steroids Further, the ability of these compounds to block the ... ileum while having little or no ... possibly by combination with ... whether or not their effects on He La cells in tissue culture ... to permeability effects is a question still to be answered Studies with labeled compounds may furnish further evidence on this point

CONCLUSIONS

1 Cortisone and certain related steroids modify electrolyte shifts in Ehrlich s ascites tumor cells

2 These same steroids cause an increase in the uptake of water by the tumor cells

3 When administered to a preparation of isolated guinea pig ileum cortisone decreases the normal muscle tone and also has definite anticholinergic actions

4 The growth of He La cells in tissue culture is significantly modified by hydrocortisone

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STUDIES IN ENDOCRINE TISSUE HOMOTRANSPLANTATION IN THE DOG UTILIZING MILLIPORE MEMBRANE DIFFUSION CHAMBERS *

I Survival of Grafts, Morphology and Fibroplasia

ROGER W HALLIN AND HENRY SWAN

Recent reports in the literature describe tissue homo and heterotransplantation involving mice rats rabbits dogs guinea pigs, monkeys and man in millipore membrane chambers [1 2 3 4] No reports to date have detailed the morphologic characteristics of endocrine tissues homotransplanted within millipore membrane between mongrel dogs or between other animals The objective of this paper is to report a study of the morphology of several dog endocrine tissues after homotransplantation in millipore membrane chambers

The theoretical basis for survival of homotransplants in millipore membrane chambers rests on the exclusion of host cells from contact with the homograft The graft lives by diffusion of tissue nutrients through the semi permeable membrane Survival of homografts in the anterior chamber of the eye up to the point of vascularization by the host is well substantiated [5] As soon as intimate cellular contact is established through vascularization the homograft rejection phenomenon occurs

A method of grafting tissues within a semidiffusible membrane permeable to tissue fluids and nutrients but excluding host cells was reported in 1932 by two Italians [6] They used collodion sacs transplanted neoplastic tissues between man and rats In 1954 Algire [1] reported his experience with millipore membranes in mice especially in the transference of neoplastic tissues

In 1952 consultation was made here with the Denver Research Institute with regard to the most likely type of membrane for use in this way A membrane manufactured from methyl methacrylate polymer (acrylic or plexiglas) [7] was recommended because of its proven low tissue reactivity We also began trying the existing membranes of Dow and Lovell (Millipore Filter Corp , Watertown Massachusetts) Several millipore membranes were tested but dog thyroid could not be made to grow or survive within the membrane In March 1955 we began studying membranes made from polyvinyl chloride manufactured by the Electric Storage Battery Company of Philadelphia Twenty different forms of membrane were tested in this laboratory over the next 2 years without achieving survival within the membrane chamber

In 1957 we began using the millipore membrane again (HA pore size 047 mm) duplicating the technique of Algire [1] This report is a study based on our initial work in dogs using a wide spectrum of glandular tissues We used dogs rather than lower vertebrates in order to more nearly simulate the tissue specificity between individuals found in man

METHOD

Normal mongrel dogs genetically unrelated to their donors were selected for this study The site of implantation of the membrane protected grafts was the paraspinal muscle mass

* From the Halsted Laboratory of Experimental Surgery University of Colorado School of Medicine Denver With the technical assistance of Brian Gordon and Robert Treasure

The chambers were constructed after the method of Algire[1] by cementing millipore membrane to one side of a 11 mm i d and 10 mm i d methyl methacrylate (plexiglas) ring 0 8 mm thick The rings were then meshed approximating the membranes the tissue having been placed between them Glue (acryloid B 7 Rohm and Haas Company Philadelphia) was used to complete the seal The membrane and tissue were kept moist with Hank s balanced salt solution penicillin and streptomycin added

The tissue to be transplanted (thyroid parathyroid ovary testis prostate adrenal cortex and later islet cells of pancreas) was removed from the donor animal placed in Hank s balanced salt then carefully sliced with a razor as thinly as possible The thin slice was then cut into 1 mm squares and these bits of tissue were placed approximately 1 to 2 mm apart on the inner aspect of an open chamber, always carefully moistened with Hank s Solution The chamber was sealed the glue allowed to set and the chamber placed deep to the lumbo-dorsal fascia in the paraspinal muscle of the host dog The wound was closed with silk and the animal was given parenteral penicillin The chambers were then removed serially during the next 60 days

Upon removal the chamber was dropped in buffered formalin the leaves of millipore membrane were separated and the tissue and membrane stained with Delafield s hematoxylin and mounted on a glass slide with the inside of the chamber face up

By microscopic examination a rough quantitation of graft survival and fibroblastic proliferation was attempted The survival of the graft was judged by cellular staining qualities and detail by preservation of normal tissue architecture and by the actual quantity of graft tissue found in the chamber taking the stain and recognizable microscopically Proliferation of fibroblasts was judged roughly as 25 to 100% depending upon the extent to which fibrous elements filled the remainder of the chamber A graph was constructed plotting the percentages so obtained against each other (Fig 1)

RESULTS

Seven different endocrine tissues were homotransplanted in millipore chambers Of these the ovary thyroid and adrenal cortex showed a rapid production of fibroblast like cells from the graft stroma Within 10 days the ovarian and thyroid grafts and within 20 days the adrenal cortex grafts within the chambers had produced a maximal fibroblastic response Survival of the grafts was inversely proportional to the fibroblastic response in 80 to 90% of the

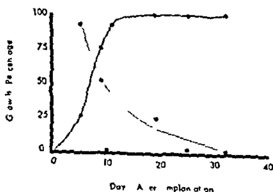


Fig 1 Rough quantitation of survival of ovarian grafts plotted against time (dotted line) and percentage of possible fibroblastic proliferation plotted against time (solid line)

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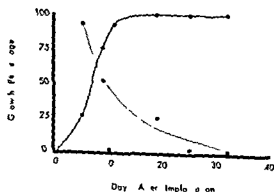


Fig 1 Rough quantitation of survival of ovarian grafts plotted against time (dotted line) and percentage of possible fibroblastic proliferation plotted against time (solid line)

individual chambers (Fig 1, ovary) In the case of the testis, the fibroblastic response was more delayed, taking 50 to 60 days to reach a maximum (see Fig 8) Accordingly, testis grafts were surviving after 20 days in 100% values and not until 35 to 50 days were graft cells diminishing in quantity to 40 to 50% of their implantation numbers The results in the case of the parathyroid and prostate were equivocal from the point of view of comparing graft survival and fibroblastic response

Most grafts survived the immediate post implantation period The implants showed an early tendency to produce fibroblast like cells from their stroma (Figs 2 and 3) No invasion of the chamber by host cells was noted in any of the preparations

Long term implants showed no viable or recognizable cells in the case of prostate, but recognizable cells were found in the case of thyroid at 150 days In the long term chamber (250 days, thyroid), the only recognizable tissue was fibrous in nature

Our controls showed early (7 to 10 days) death of free homografts of all gland tissues and prolonged survival of free autografts implanted in rectus muscle In a small group, controls of autografts in membranes showed no difference between auto and homografts (Fig 7)

DISCUSSION

Apparently the end stage of homotransplants of endocrine tissues in millipore diffusion chambers in the dog is one of fibrous tissue proliferation from the stroma of the graft, with eventual victory by the hardy fibroblasts over the more specialized endocrine cells (Fig 5) We feel it important to report this



Fig 2 Dog ovarian homograft in millipore membrane chamber at 3 days to show fibroblasts growing from the graft itself Graft in lower right corner (whole mount)



Fig 3 Dog ovarian homograft in millipore membrane chamber at 10 days after implantation showing graft in lower left hand corner and intense fibroblastic response above and to the right of the graft (whole mount)

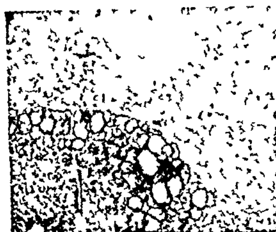


Fig 4 Dog thyroid homograft in millipore membrane chamber to show graft (lower left) and fibroblastic reaction within the chamber (upper right) at 15 days (whole mount)



Fig 5 Dog parathyroid homograft at 14 days (in lower half of figure) to show intense fibroblastic response at upper portion and to the left of figure nearly incarcerating the graft area Very few viable parathyroid gland cells remain (whole mount)

because it represents the most serious objection of this technique for long term homografting of endocrine tissues

Our early failures with millipore membrane were due largely to using too large a graft using the wrong pore size and inadequate support of the mem brane Dr Algire s plexiglas rings work well but best with nylon reinforced millipore membrane Without nylon support after a period of time the point of juncture of membrane and ring becomes weakened along the inside edge of the ring and this was a possible point of leakage We are now using rec tangular envelopes of nylon reinforced millipore membrane supported by a flat rib to prevent folding

The cause of the fibroblastic proliferation is not known it may be due to trauma in the actual transplanting process The delay in reaction of 3 to 4 days does not support this An irritative response to the millipore membrane (methyl cellulose) the glue or the plexiglas is likely, especially to the cellu lose [8] The homograft reaction appears to be side stepped but statistical proof of this fact has not been attempted in this laboratory

Preferential growth characteristics may explain the diminishing survival of graft in the face of fibroplasia In testicular tissue homografted in millipore chaml

with {

here however on study of the older slides (40 to 60 days) one sees fibroblast like cells in ever increasing proportion to the graft

Anoxia is postulated because of the increased distance of the tissue from the red blood cell The homograft oxygen supply comes from interstitial fluid

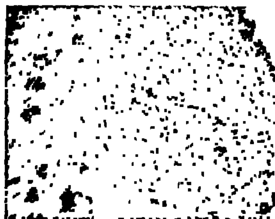


Fig 6 Dog adrenal cortex homograft at 26 days to show graft on the right and fibro blastic reaction on the left (whole mount)



Fig 7 Dog parathyroid gland autograft in millipore nylon reinforced membrane chamber at 9 days Gland is in lower left half of figure and fibroblastic cells are in upper right half of figure (whole mount)



Fig 8 Dog testis homograft in millipore membrane chamber at 19 days to show how one tissue is capable of competing with fibroblasts (whole mount)

oxygen as in the case of normal tissue, except that more membrane barriers have to be crossed

Whether or not fibroplasia affects the actual purpose of the graft apart from survival, i e , producing hormones for use in the organism, remains to be seen Hormones are produced, as shown by Sturgis and Castellanos [2] in their studies in ovarian transplants in millipore membrane chambers in rats

SUMMARY

A short history of the experience of this laboratory over the past 7 years with homografting endocrine tissues protected by semipermeable membranes is presented Histologic observations on several endocrine tissues homotransplanted in the dog over a 60 day period are detailed and discussed The marked tendency of fibroblast like elements to proliferate from the stroma of the graft is compared with the survival of the graft itself We found these two factors to be inversely related As fibroplasia increased graft survival decreased Possible reasons for the preferential growth of the fibroblasts from the graft stroma are discussed

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ENDOCRINE TISSUE HOMOTRANSPLANTATION USING THE MILLIPORE MEMBRANE*

John R Brooks Julio C Priario and Albert de Scoville

Homotransplantation of endocrine tissue has had varying degrees of success Different endocrine tissues react to homotransplantation in varying ways It appears that ovarian tissue possibly because it is less vascular, or possibly because of its multicentric cell type can be homotransplanted and survive with greater success than can other endocrine tissue Many successful ovarian homotransplants have been reported in mice and rats[1,2] Recent reports suggest that successful ovarian grafts have been obtained in man and monkey[3] An occasional adrenal homotransplant has been reported in lower animals[4] Significant homotransplantation success has not been achieved with parathyroid or thyroid in lower animals

There are no reported successful homotransplants of adrenal thyroid, or parathyroid fragments in dogs or other higher animals

The millipore membrane chamber technique of Algire[5] is well adapted to the transplantation of small minced fragments of endocrine tissue The tissue fragments are protected from host rejection by the semiporous membrane Lymphocytes and plasma cells cannot penetrate into the interior of the millipore chamber (Fig 1) Diffusion of salt glucose and protein into the chamber is possible and liberation of hormone from within the chamber into the host also occurs The success therefore of a millipore graft depends upon whether or not tissue viability and function are maintained in the new environment

METHOD

The millipore membrane is a cellulose ester of standard known pore size (0 45 micron or 0 8 micron) and thickness (150 micra) Two such circular membranes measuring 17 5 mm in diameter are attached by glue to a lucite ring at their periphery These two membranes are then glued together as a

* From the Surgical Service of the Peter Bent Brigham Hospital and the Department of Surgery Harvard Medical School Boston Supported by a Grant (A 1425) from the U S Public Health Service

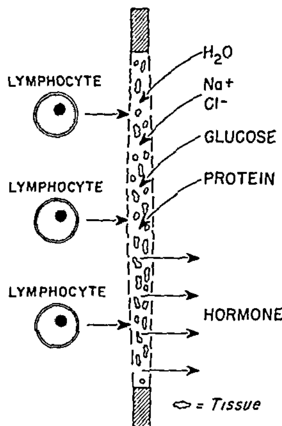


Fig 1.

protective sandwich using a lucite acetone mixture Minced fragments of tis sue to be homografted are placed within the chamber Such a 'diffusion' chamber with its contained tissue is then placed within the retroperitoneal or abdominal wall tissues of the intended recipient and allowed to remain there for varying periods of time The animal s own gland is usually removed at the same time Various studies are then carried out to evaluate function of the grafted tissue The following animal and human studies were performed

Thyroid in Rats. Rats of the Sprague Dawley strain were thyroidectomized and at a later date given radioactive iodine therapeutically to irradicate any remaining thyroid tissue Animals of this strain routinely reject skin homo transplants within the strain at a mean time interval of 13 days The thy roidectomized animals then received thyroid homografts in millipore cham bers placed retroperitoneally Six weeks later, these animals were again given radioactive iodine, this time as a tracer dose, and the following day the chambers were removed and examined under the microscope and counted for radioactivity

Thyroid in Humans. Human volunteers were subjected to normal thyroid tissue homografting into the abdominal wall musculature in millipore cham bers Six weeks later, radioactive iodine tracer studies were performed and the chamber removed and studied microscopically and for radioactive iodine uptake

Adrenal in Rats. Rats were adrenalectomized and grafted using the milli pore chamber technique Some were given autografts, others homografts

Those animals surviving this initial operation were then maintained on salt replacement for 30 days At this time salt replacement was discontinued and the animals observed The average time of death for control ungrafted adrenalectomized animals was 11 days following discontinuance of salt All rats were maintained on a standard laboratory diet

Adrenal in Dogs Dogs were totally adrenalectomized in two operations and given adrenal homografts in millipore chambers These were placed retroperitoneally in some animals in the perirenal region in others and in the abdominal wall musculature in others These animals were given long acting DOCA at the time of completion of adrenalectomy A single injection of this preparation maintained adrenalectomized animals in normal health with normal serum sodium and potassium levels for from 2 to 3 weeks Most animals succumbed at 3 to 4 weeks when the DOCA was no longer active Serum sodium potassium and corticoid levels were recorded in all animals in the postoperative period All animals were maintained on a standard Ken L-Ration diet and kept in standard laboratory cages without special environmental control At the time of death careful autopsy examination was carried out and the multiple millipore chambers removed for pathological examination A total of approximately 25 chambers were used initially containing 2 gm of tissue in each animal

Parathyroid in Dogs Parathyroid homograft studies were carried out on mongrel dogs using the millipore chamber technique Some animals underwent total parathyroidectomy and grafting at one operation others received parathyroid homografts and underwent parathyroidectomy at a later date others underwent parathyroidectomy received postoperative calcium support and then later received a parathyroid homograft All animals were maintained on a normal calcium intake in the immediate postoperative period and were followed with serum calcium and phosphorus studies and studies of renal reabsorption of phosphorus Calcium determinations were done by the method of Munson Those requiring supportive treatment were given intramuscular and/or intravenous calcium Following homografting all animals were at various intervals challenged by removing supportive calcium therapy

RESULTS

Thyroid in Rats In 43% of homografted rats 6 weeks after grafting there was a significant millipore chamber uptake of radioactive iodine when compared with muscle background Microscopic study of the tissue within the chamber in these cases revealed normal appearing thyroid gland structure and colloid (Fig 2) Thyroid stimulating hormone therapy in other animals for one week prior to radioactive iodine tracer studies failed to improve the percentage of functional takes

Thyroid in Man Millipore chambers containing normal thyroid tissue fragments were removed from human volunteers at 6 weeks Radioactive iodine in tracer doses was given prior to chamber removal To date in 8 volunteers no significant radioactive iodine uptake has been noted in these chambers In some cases microscopic evidence of viable thyroid tissue has however been obtained (Fig 3) The reaction about these chambers *in situ* has been minimal No chambers have contained evidence of host polymorphonuclear cells Some chambers have shown connective tissue suggesting survival of supporting stromal cells but not of functioning thyroid cells



Fig 2 Functioning homografted rat thyroid between two millipore membranes

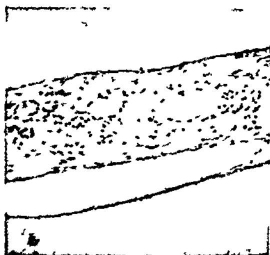


Fig 3 Homografted human thyroid showing glands and fibrosis

Adrenal in the Rat. Twenty five per cent of animals adrenalectomized and homografted survived beyond the control period A few animals have lived longer than 6 months Removal of the homograft millipore chambers in such animals has resulted in death, and microscopic examination of the tissue within the chamber has revealed viable adrenal tissue (Figure 4) Other animals surviving beyond the control period have died at a later date with chambers showing some adrenal tissue, but primarily connective tissue In these animals presumably the amount of adrenal tissue was not adequate to maintain life longer Animals dying within the control period failed to show any adrenal tissue within the chambers

Adrenal in the Dog. Control adrenalectomized dogs die immediately unless given steroid replacement Fifteen animals have been adrenalectomized and have received homografts Of these, 11 animals have died approximately 3 weeks following the last injection of long acting DOCA with extremely low serum sodium and high potassium values Three animals have survived 4 to 8 weeks after the last DOCA injection in spite of low serum sodium and high



Fig 4 Functioning homografted rat adrenal

serum potassium levels None of these animals however, has lived longer than 8 weeks beyond the last injection of DOCA Death at this later date has been associated with typically abnormal serum electrolyte levels and in many instances severe infection about the homografts

In none of the homografted animals was a significant level of serum corticoids obtained in spite of intravenous ACTH stimulation

Microscopic examination of the chambers removed at the time of death has revealed viable adrenal tissue in only one animal

Parathyroid in Dogs Sixteen animals have undergone parathyroidectomy and millipore chamber homografting Four of these animals have died in hypoparathyroid tetany Of the remaining 12 animals 3 are no longer receiving calcium support These 3 animals have normal calcium values The remaining 9 animals still require calcium replacement With the further passage of time these latter animals will again be challenged by removing calcium support in the belief that it may take longer for tissue to recover function in the millipore chamber in some animals than in others In those animals now alive without replacement therapy the millipore chambers will eventually be removed, and the animals observed Microscopic evaluation of the homografts will also be made

SUMMARY AND CONCLUSIONS

Experience with homotransplantation of adrenal thyroid and parathyroid tissue using the millipore chamber technique is reported Successful ' takes of thyroid and adrenal have been obtained in the rat Functional ' takes of adrenal in the dog have not been obtained to date although some animals have outlived the expected time of death following adrenalectomy and withdrawal of steroid support

Thyroids homografts in humans although exhibiting microscopic evidence of viable tissue in some instances have failed to show significant radioactive iodine uptake

Parathyroid homografting in dogs has been carried out and initial good functional results have been obtained in 3 animals

Although tissue *viability* and *functional ability* appear to run hand in hand following homografting in lower animals there appears to be a metabolic discrepancy between these two in dogs and humans Evidence from this work suggests that homografted tissue can be adequately protected from host interference and that the cause of failure has to do more with problems of maintaining grafted tissue metabolism In those cases where apparently normal appearing cells have been found within the millipore chamber, but in which no evidence of hormone production has been obtained it is reasonable to suppose that the millipore chamber and the surrounding fibrous barrier that it produces in the host prevents entrance of adequate nutrition into the chamber to allow hormone production or perhaps simply prevents hormone release from the chamber into the host In those animals in which function appears to exist presumably this barrier is not present

As a result of these findings this study is being extended (1) to the investigation of the metabolic requirements of various normal endocrine tissues in fragment form (2) to a study of actual diffusion potentials across the millipore membrane and the host fibrous capsule that is commonly formed around it

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INTERACTION BETWEEN HAMSTER LYMPH NODES AND HUMAN CANCERS*

LESTER P EIDELHOCH AND W BRADFORD PATTERSON

We have been investigating certain problems of tumor immunity using the heterologous system of human tumors in hamsters We are aware that the genetic differences between human cancer and hamster host are great and that resistance to transplanted tumors may have little to do with a patient s resistance to his own cancer Nevertheless it is indisputable that some type of resistance to human cancer does exist This statement is supported by clinical experience as well as by studies on serum antibodies and lymph nodes in cancer patients [1,2]

METHOD

In our studies with transplanted human cancers [3] we have found that hamsters which have supported a transplant are made resistant to reinoculation of the same tumor This resistance is a natural result of the heterograft reaction Since the lymph nodes draining the tumor bed have been implicated in this tumor immunity [4,5] we have been investigating the properties of nodes immunized against a human melanoma

If fragments of BCH 2 a melanoma are implanted under the cheek pouch mucous membrane they will grow for 4 to 6 weeks before regression occurs (Fig 1) Animals in which transplants have grown for at least a month are immune to further implants of the same tumor In our experiments we have utilized an *in vivo* technique involving simultaneous inoculation of tumor fragments and lymph nodes from immune animals into the cheek pouch In an earlier series of experiments we were unable to demonstrate any tumor cytotoxic effect in serum from resistant animals or in lymph nodes implanted into the peritoneal cavity

In the present study transplants are made into both cheek pouches of a series of immature hamsters These animals are given 2 5 mg of cortisone subcutaneously twice a week for 6 doses A group of animals without tumors are treated with cortisone in the same manner and serve as donors for control lymph nodes

* From the Department of Surgery Harvard Medical School the Fifth Surgical Service and Sears Surgical Laboratory Boston City Hospital Aided by a grant from the American Cancer Society



Fig 1 Patient from whom melanoma was removed and healthy transplant a year later after 17 passages in hamsters

After one month when the tumors have begun to regress the superficial cervical lymph nodes of all animals are excised split, and immediately implanted into the cheek pouch of a second hamster alongside a new fragment of the transplantable human melanoma By this means lymph nodes and tumor fragments are exposed in close proximity in a medium very favorable for growth of these tissues The new host hamster is treated with cortisone in order to depress his own reaction to the two foreign tissues

At intervals of 24 and 48 hours the cheek pouch is biopsied and slides made of the implants

DISCUSSION

The typical host reaction to implantation of the tumor alone in a cortisone treated animal is mild There is edema and infiltration by polymorphonuclear leucocytes Central areas of ischemic degeneration are seen but no widespread necrosis occurs and peripherally the tumor remains healthy

Control transplants employing only lymph nodes also elicit little reaction Some invasion by polymorphonuclear cells occurs and the lymphocytes migrate or lyse within a week leaving only the reticular framework

With tumor and control lymph nodes implanted together the results do not differ greatly from those obtained if these fragments are implanted separately At 24 hours some necrosis of tumor is seen and there is edema of the pouch with a cellular infiltrate (Fig 2a) At 48 hours most tumor cells are viable and active and the lymph node is emptying

In the pouches containing resistant lymph nodes and tumor fragments an entirely different picture is present (Fig 2b) In 24 hours there is a violent reaction characterized by infiltration of the tumor with lymphocytes plasma cells and polys The stroma is very edematous with blood vessel dilatation At 48 hours the tumors are often wholly necrotic and whatever tumor remains shows marked edema The tumor and node are both infiltrated with lymphocytes and histocytes Stromal blood vessels are surrounded by cuffs of mononuclear cells which form a dense infiltrate in the edematous active connective tissue At 4 days most of these pouches show only an area of vigorous healthy granulation tissue to mark the site of previous acute reaction

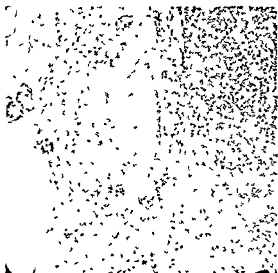


Fig 2a Normal cervical lymph node adjacent to tumor fragment, 48 hours after implantation



Fig 2b Resistant cervical node and tumo fragment, 48 hours after implantation

CONCLUSIONS

We conclude from these experiments that in hamsters which have bee made resistant to this tumor, the regional lymph nodes exert a cytotoxic actio on the tumor. This activity is not possessed by control hamster lymph node

Although the secondary host has been treated with cortisone to depres cellular response, we cannot be certain whether the infiltrating mononuclea cells come from the implanted resistant lymph node or from the new hos Further studies with secondary hosts which have been prepared by heav x-radiation to destroy lymphoid tissue will help to clarify this point.

We believe that this reaction is a manifestation of transplantation immu nity. As such it may be related only to the genetic differences between th human tumor and hamster host. We intend to pursue the study, however, ir order to determine, if possible, existence of tumor-specific factors

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MELANOMA INHIBITION IN HAMSTERS AND MICE BY ORTHO META AND PARA PHENYLENEDIAMINE *

VERNON RILEY AND JOSEPH G FORTNER

Inhibition of the growth rate of transplanted hamster and mice melanoma has been observed in animals treated with ortho meta and para phenylenediamines [1-2] The chemotherapeutic trial of these compounds in animals was stimulated by the observation that a synergistic oxygen consuming reaction occurs *in vitro* between PPDA (para phenylenediamine) and dopa (dihydroxyphenylalanine) when the two purified compounds are combined in a Warburg manometric apparatus [3, 4] Since one of the reactants dopa is an accepted precursor of melanin and is presumed to be present in the melanoma cell [5] the possibility of generating a specific intracellular reaction in this malignant tissue appeared theoretically feasible It was reasoned that if such a reaction took place inside the melanoma cell between the administered phenylenediamine and the endogenous dopa its avid oxygen consumption might deprive the neoplastic cell of its normal oxygen requirements and thereby interfere with its essential metabolism An insoluble pigment is also produced by this reaction which could in addition constitute a trapped toxic substance capable of further embarrassing the melanoma cell Also of possible pertinence are the inhibitory effects of the phenylenediamines against certain enzymes [6]

Although the above working hypothesis has not been established the following data illustrate preliminary findings in the exploration of such an approach the special usefulness of the transplanted melanoma in the development of a rational attack and some of the practical possibilities for exploitation of a metabolic difference found in a specific tumor type

METHOD

Th

No

a Syrian Golden hamster The histological and biological characteristics have been previously described [7, 8] The Cloudman S91 mouse melanoma [9] was carried in DBA 2 inbred and DBA 2 hybrid mice Ortho meta and para phenylenediamines (OPDA MPDA PPDA) were used as the dihydrochloride or the free base The latter was preferred where equal purity with the dihydrochloride salt could be obtained Samples of the compounds employed were obtained from the following companies Eastman Paragon Testing Laboratories Matheson Coleman & Bell and Abco Chemical Company Dopa (3 4 dihydroxyphenyl DL alanine) was from Nutritional Biochemicals Corp Oxygen consumption measurements associated with the *in vitro* experiments employed standard manometric techniques [10] with the reactions run in air at 37 or 38°C and the vessels shaken at 112 cycles per minute at an amplitude of 4 cm Reagent concentrations in the vessels are expressed in molar terms and

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* From the Division of Experimental Chemotherapy Sloan Kettering Institute for Cancer Research and the Department of Surgery Memorial Center for Cancer and Allied Diseases
Supported in part by a grant from the National Institutes of Health U S Public Health Service With the assistance of Elisabeth Booth Alice Gale Vincent Nole and members of the Volunteer Department of Memorial Center

were calculated as final concentrations following tipping of the sidearm components All labile or oxidizable reagents were prepared shortly before being added to the vessels or injected into animals Hydrogen ion concentrations were appropriately controlled and measured by a glass electrode pH meter Further details of procedures are given in the description of the experiment or are contained in the figures

RESULTS

Our initial efforts to obtain melanoma inhibition with intraperitoneal injections of PPDA were essentially negative This was apparently due to the inability to establish a sufficiently high level of the drug without serious toxic effects However, by administering the compound through the drinking water where it was consumed in small amounts on a continuous basis the total daily consumption was greatly increased The maximum dose permissible by the rapidly adsorbing intraperitoneal route was approximately 40 mg /kg /day whereas the *ad libitum* consumption through the drinking water was estimated at approximately 100 gm /kg /day

Figure 1 illustrates the inhibition of growth of the Cloudman S91 mouse melanoma when PPDA was added to the drinking water at a concentration of 150 mg /100 ml In this experiment the tumors were well established since they had been permitted to grow for 27 days before treatment began There were 10 mice in each group at the time treatment started and each point represents the average tumor volumes of the group It may be seen that there was approximately a one third increase in survival time and somewhat more than 60% inhibition of growth of the established tumor Although this compound has been considered carcinostatic rather than carcinolytic the suggestion of partial regression may be real since the effect was noted in several of the tumors of this group (Fig 1)

Since a difference among the para meta and ortho isomers had been observed in the rates of manometric oxygen consumption with dopa, it seemed

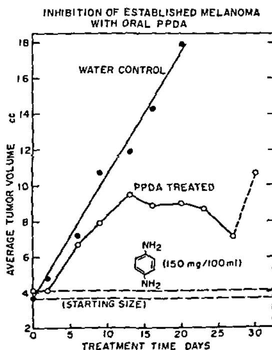


Fig 1 Inhibition of established mouse melanoma with oral *p* phenylenediamine (PPDA) (PPDA 150 mg /100 ml distilled drinking water tumor 27 day post implant of Cloudman S91 melanoma in DBA 2 females)

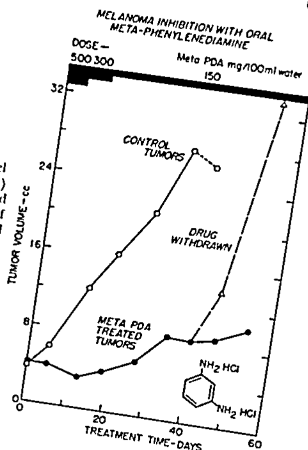


Fig 2 Inhibition of established mouse melanoma with m phenylenediamine (MPDA) 500 to 150 mg/100 ml distilled drinking water, 19 day post implant of Cloudman S91 melanoma in DBA 2 hybrid males)

appropriate to determine whether there were any correlations in their antitumor properties The somewhat increased tumor inhibition obtained with MPDA consequently came as a surprise since the oxygen consuming reaction rate of this compound with dopa had been considerably lower than that of PPDA Figure 2 illustrates the relative melanoma inhibition and the dose schedule administered over a period of 60 days Since the MPDA in acute doses is less toxic than PPDA, attempts were made to increase the therapeutic dose This is shown at the top of Figure 2 The experiment was started at 500 mg/100 ml of drinking water but since weight loss and deaths were encountered the dose was reduced to 300 mg/100 ml and, eventually, to 150 mg/100 ml, which the animals tolerated A point of interest in this experiment is illustrated by the broken line Following 40 days of treatment, the drug was withdrawn from 2 of the mice with consequential rapid growth of the released tumors, as shown This compound was employed in the drinking water in the dihydrochloride form without neutralization (Fig 2)

A similar experiment was designed to test the relative inhibitory properties of OPDA The primary difference in this experiment was the route of administration Since OPDA was found to be considerably less toxic in acute doses than either PPDA or MPDA, intraperitoneal injections were again tried Figure 3 illustrates the resulting substantial inhibition that was obtained with the Cloudman S91 melanoma when treated with OPDA over a 70 day period The extension of survival time of the treated animals and their comparative weights are also illustrated in the figure The increase in weight of the saline control group reflects the increased weight of the rapidly growing tumor in contrast to the relative constant weight of the treated mice It may be noted that there is a correlation between the upward inflection in the weight

MELANOMA VOLUMES AND ANIMAL WEIGHTS DURING o-PHENYLENEDIAMINE ADMINISTRATION

Fig 3 Influence of o phenylenediamine (OPDA) on melanoma growth rate animal survival time and average mouse weights during prolonged intraperitoneal administration (OPDA 100–200 mg/kg/day 17 day post implant of Cloudman S91 melanoma in DBA 2 mice)

curve and the tumor growth of the treated mice between the twentieth and thirtieth day The subsequent tumor regression and the return of the mean weights to normal is probably real since it has been duplicated in independent experiments (Fig 3)

The hamster melanomas [7, 8] are of special interest and value experimentally because of their similarity to the human lesion, including early and extensive metastasis, similarity of histology, and origin in junctional nevi The inhibitory effects of OPDA on one of the hamster melanomas the Melanotic Melanoma No 2 (H M M 2), is illustrated in Figures 4 and 5 The differential increase in average tumor volume between the treated and control animals is shown over a period of 41 days, with the OPDA dose in mg/kg of hamster per day indicated by the stippled scale The drug was given intraperitoneally and intermittently, as shown Although the usual treatment schedule in such experiments is 5 consecutive days of injection with a 2 day recess over the weekend, one day was skipped in the middle of the first 2 weeks

HAMSTER MELANOMA VOLUMES DURING OPDA TREATMENT

Fig 4 Inhibition of hamster melanoma with OPDA (OPDA 200–250 mg/kg/day 1 day old H M M 2 in female Syrian Golden hamsters)

Fig 5 Control and OPDA treated hamster melanomas after 29 days (Tumors from experiment illustrated in Figure 4)

of treatment in this experiment. Figure 5 shows a photograph of dissected tumors removed on the twenty-ninth day of treatment from 5 representative animals in each experimental group. The relative difference in size represents an 83% inhibition based on the average weights of the two tumor groups at this point in the experiment. Although in most experiments the inhibited tumors eventually killed the hosts, or the animals died from accumulated toxicity effects of the drug, or a combination of both, instances of total regression of tumors under treatment have been observed. When such animals were subsequently challenged by an attempt at reimplanting the tumor, the animals were found to be resistant and the tumor failed to grow.

Figure 6 illustrates the oxygen consuming reaction obtained when PPDA is combined with dopa as compared with its analogs, tyrosine and phenylalanine. OPDA and MPDA give a similar reaction but at a considerably lower rate. These manometric reactions take place at physiological temperatures with the pure compounds and in the absence of tissue extract or enzymes of any sort. The relative specificity of the reaction is indicated by the finding that only 6 or 7 compounds were active in this manner with dopa out of 150 diamino compounds tested. On the other hand, several compounds closely related to dopa are active with PPDA. It is possible that some of these may be responsible for the activity observed when PPDA is combined with the tumor homogenate, blood plasma, or urine of advanced, pigmented melanoma patients.

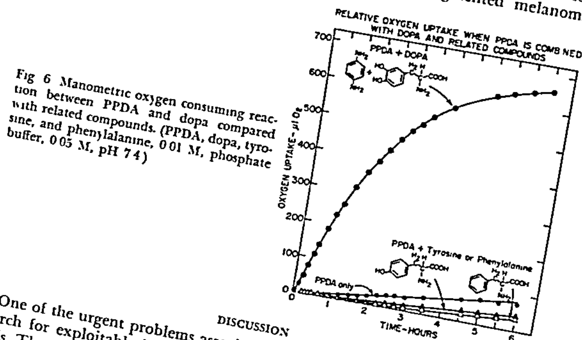


Fig 6 Manometric oxygen consuming reaction between PPDA and dopa compared with related compounds. (PPDA, dopa, tyrosine, and phenylalanine, 0.01 M, phosphate buffer, 0.05 M, pH 7.4)

DISCUSSION

One of the urgent problems associated with current cancer research is the search for exploitable biochemical differences between normal and cancer cells. The presence of naturally occurring, unique metabolites that accumulate in the malignant cell as an expression of a subtle difference in metabolism is one of the obvious possibilities. The melanoma is a conspicuous example of a malignancy where such a difference could be expected. The evidence at hand suggests that the tyrosine metabolism of this tumor is "faulty," or different, in that some relatively unique products are produced rather than the normal physiological tyrosine products such as homogentisic acid, thyroxine,

tyramine, and adrenaline Although the explanation of the melanoma inhibition is tentative, it is consistent with the finding that the S91 melanoma can "protect its host from acute toxic challenge by PPDA [11] A logical extension of the speculative aspect of these studies involves a search for analogous unique metabolites in other tumors which can be reacted with suitable exogenous agents One nonmelanoma, the Ehrlich carcinoma, is inhibited by OPDA, this raises interesting questions concerning the metabolism of this tumor and of alternative mechanisms of the reaction

It was unexpected to find that melanoma inhibition increased from the para to meta to ortho forms of the compound However, the toxicity of these isomers decreased in the same order, thus permitting the administration of correspondingly higher doses, which may account for the differential effectiveness

SUMMARY

Inhibition of hamster and mouse melanomas has been obtained with ortho-, meta, and para phenylenediamine which have also been shown to react with dopa or other melanoma components in manometric experiments Possibilities of the interrelationship of these observations are discussed

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THE DEVELOPMENT OF THYROID CANCER AND OTHER ABNORMALITIES IN SYRIAN HAMSTERS MAINTAINED ON AN IODINE DEFICIENT DIET *

JOSEPH G FORTNER PHYLLIS A GEORGE, AND STEPHEN S STERNBERG

This paper reports, for the first time the occurrence of cancer and hyperplasia of the thyroid gland in the Syrian Golden hamster fed a diet deficient in iodine It is already well known that the incidence of thyroid cancer in man is highest in regions of the world where the soil is deficient in iodine [1]

* From the Experimental Oncology Section of the Division of Experimental Surgery of Sloan Kettering Institute Andre and Bella Meyer Laboratories and the Department of Surgery Memorial Center New York City Supported by grants from the National Institutes of Health U S Public Health Service With the invaluable assistance and support of Drs H F Randall and I S Ravdin and the technical assistance of Miss Alice Gale

Cancer has been produced in rats and mice by prolonged treatment with antithyroid compounds and radioactive iodine.[2,3,4,5,6] The hamster cancers are particularly interesting since they seem to have been induced by an iodine deficiency and metastases have been observed only in females.

METHOD

Fifty male and 50 female, young adult, Syrian Golden hamsters were obtained from a commercial dealer. They were stabilized for 17 days in the laboratory on a diet of Purina Laboratory Chow and water *ad libitum*, with supplements of sunflower seeds and carrots. At initiation of the experiment, the hamsters were grouped so that two of the same sex were housed together in a steel cage with a wire mesh bottom. At this time, the weights of the males varied from 71 to 120 gm. with a mean of 91.1 gm. The females weighed 75 to 133 gm. with a mean of 97.0 gm. The diet was changed so that it consisted only of unpolished brown rice with one thin slice of fresh carrot per animal (approximately 1 gm.) as a daily supplement. Tap water was given *ad libitum*. Sample analysis of the diet revealed that the rice and carrots contained 0.231 gamma % and 0.007 gamma % iodine, respectively. The animals' daily food and water intake was not measured. Sixty-one of the animals were injected with human bile but the details are not included as this factor is irrelevant to the currently reported observations.

The animals were allowed to live out their life span or were sacrificed when moribund. At death, a complete necropsy was performed. All grossly observed abnormalities as well as routine sections of viscera were examined microscopically. The thyroids were weighed and measured. Some animals were partially eaten or autolysed and are therefore deleted in calculating the results.

Another experiment was carried out to further evaluate the effects of an iodine deficient diet. Thirty two weanling hamsters (16 males and 16 females) were fed the above dietary regime for 6 weeks. As a control, 32 comparable animals were placed on a diet of Purina Laboratory Chow. At the end of the 6 week period, the surviving 18 animals were anesthetized with intraperitoneal nembutal. Blood was drawn from the inferior vena cava for analysis of its serum total and protein bound iodine content. Pooling of blood from 3 to 4 animals of the same sex was necessary in order to obtain sufficient serum for the chemical determinations. The iodine values were determined by the method of Zak *et al.*[7] The thyroid glands of 5 male and 5 female animals from each of the two groups were selected at random for gross and microscopic study.

RESULTS

The survival times of the 100 hamsters on the iodine deficient diet varied from 36 to 685 days with a mean of 441 days. Males survived from 36 to 712 with a mean of 406 days and the females from 42 to 785 with a mean of 474 days.

The weights of the animals were less at time of death than at the beginning of the experiment. The weights of males varied from 37 to 92 gm. with a mean of 52 gm. A similar range of 37 to 92 gm. with a mean of 58 gm. was present in the females.

The severity of thyroid abnormalities varied directly with the time the animals had been on the iodine deficient diet. However, no animals died prior to 36 days. Changes thereafter were progressive for the first 300 days,

after which time, the thyroids showed considerable variation in their pathological features Grossly, the thyroids were reddish brown and symmetrically enlarged Nodularity of the glands was usually noted with a frequency which increased with the age of the animal Microscopically, the earliest change noted was a uniform and easily detectable increase in the height of the follicular epithelium This was associated with a diffuse and symmetrical enlargement of the follicles With advancing age variations in the size and shape of the follicles occurred and small papillary projections were noted In localized areas, collections of follicles developed which were characterized by a lack of uniformity in size and shape Some were markedly dilated and cystic with flattened epithelium, in others, papillary projections were prominent Hyperchromatic nuclei were found in some follicles

Nine of 49 females (18%) available for study were found to have metastatic thyroid cancer They had been on the iodine deficient diet from 393 to 674 days, with a mean of 520 days Pulmonary metastases were grossly apparent in 2 An additional 6 females were found to have metastases on routine microscopical examination of selected portions of each lobe One female was found to have a metastatic cervical lymph node deposit but no pulmonary metastases

In those animals which showed metastases, all of the previously described changes *in the thyroid were fully developed However, in no instance* was there a degree of atypia or anaplasia which is conventionally associated with cancerous changes either in the thyroid or in the metastases In other words, the changes were those usually described as 'metastasizing struma" The metastases were all of the follicular type of carcinoma, no papillary formations were seen outside the thyroid gland In the thyroid itself, papillary projections were seen, as previously noted, but these were diminutive and of the type seen in hyperplasia Because of the lack of significant anaplasia in the thyroid tumors, difficulty was encountered in diagnosing cancerous changes in the absence of metastases All the lung metastases were of the follicular type of carcinoma of the thyroid In all cases the metastases were located near the periphery of the lungs The incidence of metastases may have been, and in all probability was, greater than is apparent for serial sectioning of the lungs was not done No other sites of metastatic tumor were found None of the males demonstrated thyroid metastases

Iodine estimations on 25 animals fed the iodine deficient diet for 6 weeks and on their controls are shown in Table 1 The mean serum total iodine

Table 1 Comparative Thyroid Weights, Serum P B I, and Total Iodine Values Iodine Deficient Diet for Six Weeks

ANIMALS		THYROID WEIGHTS (MG)		TOTAL IODINE (μg %)		PROTEIN BOUND IODINE (μg %)	
NO	GROUP	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
13	Male Exp	11 1–16 9	13 8	1 4–2 8	2 1	0–2 7	1.5
13	Male Control	5.2–12 4	8 3	5.5–11.5	9 9	1 0–2 0	1.5
12	Female Exp	10 1–15 0	12.5	1 4–2.2	1 8	0 9–2 0	1.5
10	Female Control	3 4–6 0	4.8	3 4–3 7	3.5	0 7–1 4	1.5



FIG 1 Gross appearance of thyroid carcinoma in a female hamster An enlarged paratracheal lymph node and pulmonary metastases are also evident

values in the controls was 9 9 gamma % for the males and 3 5 gamma % for the females This contrasted with that of the experimental group which were found to have 2 1 gamma % for the males and 1 8 gamma % for females A mean P B I of 1 5 gamma % was found in both males and females of test and control groups In this experiment the thyroids of male control animals had a mean weight of 8 3 mg in contrast to male test animals which had a mean weight of 13 8 mg Corresponding values in the females were 4 8 mg and 12 5 mg respectively The beefy red color of the enlarged thyroid glands of the test group was prevented in an additional group of animals, by administering 1 to 2 gamma of iodine as sodium iodide daily by subcutaneous injection

DISCUSSION

Evaluation of the experimental data is made particularly difficult by the histologic response of the hamster s thyroid gland to the iodine deficient diet Under the conditions described thyroid cancers could be diagnosed with certainty only when associated with metastatic spread These tumors were well differentiated follicular carcinoma and conformed to what has been termed benign metastasizing struma in man Other thyroids with a similar histologic appearance but without demonstrable metastases were classified as benign In addition some glands without associated pulmonary metastases appeared more dedifferentiated than those with metastases We are unwilling to classify these as cancer at this time Obvious hyperplasia as the sole

abnormality was also observed These considerations led to a tentative diagnosis of thyroid hyperplasia in 47 animals and possible cancer without metastases in 39 animals No sex predilection was found for these lesions However definite thyroid cancers (with metastases) were found only in females Nine of 19 females (18%) had either pulmonary or lymph node metastases The involved lymph node was widely separated from the thyroid gland Pulmonary metastases were peripheral in location

The reason for the occurrence of metastases only in females is not evident Chance selection in a relatively small group of animals is possible Hormonal influence may be implicated directly or indirectly It is noted (Table 1) that the serum total iodine values of females and males is essentially the same after 6 weeks on the iodine deficient diet Normal values were found to be considerably higher in males than in females The serum iodine levels of hamsters fed the iodine deficient diet in long term experiments is unknown The iodine content of our diet is lower than that usually employed i e by Money and Rawson[2] in rat experiments Other nutritional deficiencies in the rice and carrot diet are undoubtedly present

Spontaneous thyroid cancers have been observed in hamsters maintained on a standard laboratory diet in our laboratory However the gross and microscopic appearance of these tumors differ from those present in animals fed the iodine deficient diet Further studies are in progress

SUMMARY

Thyroid carcinoma with metastases was found in 18% of female Syrian Golden hamsters fed a diet deficient in iodine Metastatic thyroid cancer was not observed in any of the males The proved thyroid cancers were so well differentiated as to preclude the definite diagnosis of cancer in other animals which had no demonstrable metastases

Spontaneous thyroid cancer has been observed in hamsters fed a standard laboratory diet in our laboratory

Iodine deficiency appears to be a casual agent for some thyroid cancers in the hamster Serum total and protein bound iodine values for the hamster are presented

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THE ACTION OF 3, 5, 3 L-TRIIODOTHYRONINE AND NITROGEN MUSTARD ON THE "TAKE" OF THE WALKER 256 RAT TUMOR *

LOREN J. HUMPHREY, EVERETT T. HOPPE, AND FREDERIC A. DEPEYSTER

Thyroxin has been reported to inhibit the growth of the Walker 256 rat tumor.[1] Other reports have shown the inhibitory effect of the hyperthyroid state against various neoplasms of mice and rats. Previous work in this laboratory has demonstrated the effectiveness of nitrogen mustard in decreasing the "take" of Walker 256 cells injected into the portal vein of rats.[2] The purpose of this study was to determine whether the hyperthyroid state would enhance or reduce the antitumor action of nitrogen mustard against the "take" of Walker 256 cells.

METHOD

Female Sprague-Dawley rats weighing between 150 and 170 gm. were used in this study. Three sets of rats received a subcutaneous saline solution of 3, 5, 3 L-triiodothyronine, (Smith, Kline, and French Laboratories), 200 μg./kg., 25 μg./kg., and 5 μg./kg. respectively, administered daily for 3 days prior to the inoculation of the tumor cell suspension and continued for 4 days thereafter.

On the third day of triiodothyronine therapy a celiotomy with aseptic technique was performed under intraperitoneal nembutal anesthesia. A sterile preparation of the Walker 256 tumor cell suspension (25,000 cells/cc.) modified after Lucke's technique was aged 6 to 12 hours at room temperature before intraportal injection of 1 cc. A 50 to 70% "take" in control animals was yielded by this method.

Nitrogen mustard (Merck) (0.5 mg./kg.) was injected into the portal vein immediately after the cells in Groups 1 and 2. In Group 3 nitrogen mustard was injected intraportally *one hour after* the cell suspension. This delay of one hour was necessary to decrease the effectiveness of nitrogen mustard which, when given immediately in Group 2 destroyed cells aged 12 hours so effectively that there were no "takes" with nitrogen mustard alone or in combination with triiodothyronine. Alternate animals served as controls throughout the experiment. Animals dying within 7 days of operation were excluded from this study. All other animals that died within 20 days or were killed 21 days after cell inoculation were examined for the presence of tumors.

RESULTS

In the first experiment, those rats that had received triiodothyronine (200 μg./kg.) for 3 days weighed on an average 13.5 gm. less than the average control rats. There were 94% "takes" in control rats and 100% in rats receiving triiodothyronine. Only 20% of those rats receiving nitrogen mustard had "takes" contrasted to 56% when triiodothyronine was used with nitrogen mustard (Table 1).

In the second experiment, rats that had received triiodothyronine (25 μg./kg.) for 3 days weighed on an average 10 gm. less than the average control

* From the Department of Surgery, University of Illinois College of Medicine, Chicago. Aided by a grant from the United States Public Health Service CY-3482.

Table 1 The Percentage of Takes of Walker 256 Carcinoma Cells (25,000 aged for 6 hours) Injected Intraportally and Treated by 3, 5, 3 L triiodothyronine (200 μg /kg) and Nitrogen Mustard (0 5 mg /kg Given at Time of Cell Injection)

GROUP	NO OF ANIMALS	% TAKES
1 Tumor	17	94
2 Tumor + Triiodothyronine	13	100
3 Tumor + Triiodothyronine + Nitrogen Mustard	16	56
4 Tumor + Nitrogen Mustard	19	20

rat There were 30% 'takes' in control rats and 15% "takes" in rats receiving triiodothyronine Nitrogen mustard alone and with triiodothyronine was so effective with this concentration of aged cells that there were no "takes" in either group (Table 2)

Table 2 The Percentage of Takes of Walker 256 Carcinoma Cells (25,000 aged 12 hours) Injected Intraportally and Treated by 3, 5, 3 L triiodothyronine (25 μg /kg) and Nitrogen Mustard (0 5 mg /kg Given at Time of Cell Injection)

GROUP	NO OF ANIMALS	% TAKES
1 Tumor	20	30
2 Tumor + Triiodothyronine	18	15
3 Tumor + Triiodothyronine + Nitrogen Mustard	18	0
4 Tumor + Nitrogen Mustard	20	0

Those rats that received triiodothyronine, 5 μg /kg, for 3 days (experiment 3) weighed 3 5 gm less than the average control rat There were 74% 'takes in control rats and 58% 'takes in rats treated with triiodothyronine Rats treated with nitrogen mustard alone had 2% 'takes' and when combined with triiodothyronine there were 10% 'takes' (Table 3)

Table 3 The Percentage of Takes of Walker 256 Carcinoma Cells (25,000 aged 12 hours) Injected Intraportally Treated by 3, 5, 3 L triiodothyronine (5 μg /kg) and Nitrogen Mustard (0 5 mg /kg given One Hour after Cell Injection)

GROUP	NO OF ANIMALS	% TAKES
1 Tumor	44	74
2 Tumor + Triiodothyronine	43	58
3 Tumor + Triiodothyronine + Nitrogen Mustard	40	10
4 Tumor + Nitrogen Mustard	44	2

DISCUSSION

Fewer "takes" were observed in rats receiving 25 and 5 µg./kg. triiodothyronine than in control rats. When a massive dose of triiodothyronine (200 µg./kg.) was used, there were more "takes" suggesting a decrease in the tumor resistance due to the toxic effect of the drug. The favorable results in groups receiving less toxic doses are consistent with the findings of many other studies that have reported inhibition of tumor growth by thyroid compounds. It has been shown that thyroid compounds given for a short time interval deplete the adrenals.[3] The slight antitumor effect seen with triiodothyronine may be due to adrenal depletion which prevents the stress reaction of celiotomy from increasing tumor "takes."[4]

Triiodothyronine in combination with nitrogen mustard (Tables 1 and 3) was less effective than nitrogen mustard alone in preventing takes, suggesting an antagonistic action between these drugs. This antagonism may result from either a decrease in the resistance of the rat to the cancer cell due to the toxic effect of triiodothyronine (most pronounced at 200 µg.), or it may result from a more rapid destruction of nitrogen mustard due to the increase in cellular metabolism associated with the hyperthyroid state.

We are now investigating the effect of the hypometabolic state on the anticancer action of nitrogen mustard.

SUMMARY

An experiment has been conducted to determine the effect of the hyperthyroid state on the anticancer action of nitrogen mustard. All rats received 25,000 aged Walker 256 carcinosarcoma cells intraportally. Subcutaneous injections of *3, 5, 3 L-triiodothyronine* were *given to one-half the rats 3 days* prior and 4 days after tumor cell inoculation. One-half of these rats received nitrogen mustard intraportally at the time of tumor cell inoculation. Alternate rats served as controls and all animals were sacrificed 21 days after cell inoculation and examined for "takes."

Triiodothyronine, 5 µg./kg., *decreased* "takes" (58% vs. 74% in controls).

Triiodothyronine, 25 µg./kg. *decreased* "takes" (15% vs. 30% in controls).

Triiodothyronine *increased* "takes" when given with nitrogen mustard (10% with 5 µg. vs. 2% in controls and 56% with 200 µg. vs. 20% in controls).

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LACTATION INDUCED BY LUTEOTROPHIN IN WOMEN WITH METASTATIC CANCER OF THE BREAST *

I *The Effect of Adrenalectomy and Hypophysectomy on Lactation*

THOMAS L DAO

The effects of luteotrophin on the breast of women postpartum has been extensively investigated, but otherwise few observations have been made in the human Huggins and Dao[1] reported in 1954 that many patients with carcinoma of the breast, even though in the 6th and 7th decade, secreted milk when luteotrophin was administered, despite the menopause and earlier removal of ovaries Lactation thus induced often persisted for many months even in the absence of adrenals and gonads Huggins[2] also observed that most dogs with spontaneous mammary cancer possess lactation, and this characteristic persisted for many months despite the removal of the adrenals and gonads

This study presents evidence that the anterior pituitary gland plays an important role in the initiation and maintenance of lactation in man The significance of induced lactation in certain women with mammary cancer will also be described

METHOD

Luteotrophin (E R Squibb) dissolved in physiological saline was injected subcutaneously in daily amounts of 500 I U for 10 days to 11 women with metastatic cancer of the breast The mammary secretion was considered as milk when 1) the white opaque fluid was crammed with fat droplets without leucocytes other than colostrum corpuscles, 2) biopsy of breast tissue stained with Sudan III showed secretion in the alveoli, and 3) the identification of the milk sugar (lactose), in the secretion by paper chromatography All these patients subsequently underwent bilateral adrenalectomy After one week following the surgical procedure the final maintenance dosage of cortisone acetate (50 mg /daily) was instituted and continued in all the subjects

RESULTS

Lactation, when it occurred, varied from a few drops to a few cc daily from the breast (Fig 1) It usually occurred on the 7th to 10th day of injection

Lactation in Women with Mammary Cancer. Six of the 11 patients lactated following the administration of luteotrophin Menstruation had ceased in all, 5 had surgical castration earlier and one had irradiation to the ovaries Three patients had previous hormonal therapy prior to adrenalectomy (Table 1) Biopsy of breast was performed in each of these cases and histological examination revealed sudanophilic fat droplets in the alveolar cells and in the lumina of the ducts (Fig 2) Milk secretions in five patients

* From the Breast Service Department of Surgery Roswell Park Memorial Institute

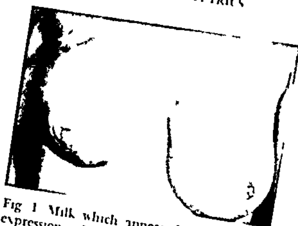


Fig 1 Milk which appeared following the expression of the breast in a castrated woman age 37 with mammary cancer following the injection of luteotrophin for 10 days



Fig 2 Fat droplets in the alveoli and in the lumina of the ducts of the breast in a woman with lactation after luteotrophin

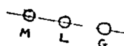


Fig 3 Paper chromatographs of the mil[k] Note milk spot corresponds to lactose M indicates milk L indicates lactose and G in[di]cates glucose

were chromatographed on paper and in all these cases lactose was identified (Fig 3)

All the patients subsequently underwent bilateral adrenalectomy In no instance did lactation cease following the removal of the adrenal glands An attempt to suppress the persistent lactation in 4 patients was made with steroidal hormones but none of these measures were efficacious Patients A D and L M were given testosterone propionate 50 mg daily for 2 months and the secretion of milk was not stopped Patient N W received diethyl stilbestrol 15 mg daily for 30 days and lactation continued Patient A L was given progesterone 100 mg daily for 40 days and lactation was not influenced

While the lactation persisted the disease continued its unfavorable progress None of these patients who had lactation following luteotrophin treatment responded to adrenalectomy

Hypophysectomy was performed in 3 patients 3 4 and 7 months following adrenalectomy In all 3 patients lactation ceased between one to two weeks after the operation Their cancers however were not influenced by this surgical procedure and all three patients succumbed to the disseminated disease 2 3 4 months after hypophysectomy

Table 1 Response to Adrenalectomy and Hypophysectomy in Patients Who Lactate Following Luteotrophin Administration

CASE & AGE	MENOPAUSE AGE	CAUSE OF MENOPAUSE	HORMONAL TREATMENT DURATION	DURATION OF LACTATION	RESPONSE TO ADRENALECTOMY	RESPONSE TO HYPOPHYSECTOMY
V D 15	45	Surgical Castration	None	4 months	none	none (lactation ceased)
N W 36	36	Surgical Castration	None	7+months	none	none (lactation ceased)
A L 15	11	Surgical Castration	Testosterone 3 months	4 months	none	
I M 37	35	Surgical Castration	Estrogen 3 months	4+months	none	none (lactation ceased)
K A 19	48	X ray Castration	Testosterone 9 months	6+months	none	
S W 42	10	Surgical Castration	None	2 months	none	

Failure of Certain Women with Mammary Cancer to Lactate Following Luteotrophin. There were 5 patients in this category (Table 2) Menstruation had ceased in all 5 patients Following adrenalectomy, 4 of the 5 patients had objective regression of cancer to some magnitude All 4 patients are now living and well with no evidence of relapse of disease Patient G M showed subjective improvement for 6 months after adrenalectomy but at no time was any objective regression demonstrated

Failure to Induce Lactation in an Ovariectomized, Adrenalectomized and Hypophysectomized Woman by Luteotrophin. This patient, L M, having prolonged lactation after luteotrophin administration, was submitted to hypophysectomy 3 months after adrenalectomy since this surgical procedure failed to produce remission of the disease Lactation stopped completely 10 days after hypophysectomy Six weeks later, another course of luteotrophin was given to her but no lactation was observed

Table 2 Response to Adrenalectomy in Patients Without Lactation Following Luteotrophin Administration

CASE & AGE	MENOPAUSE AGE	CAUSE OF MENOPAUSE	HORMONAL TREATMENT TYPE AND DURATION	RESPONSE TO ADRENALECTOMY
H T 49	47	Surgical Castration	none	Objective Remission
A T 51	50	Surgical Castration	Testosterone	Objective Remission
M S 52	49	X ray Castration	Testosterone 10 months	Objective Remission
G M 66	51	Spontaneous	Estrogen 3 months	Subjective improvement 6 months
A L 60	42	Spontaneous	Estrogen 6 months	Objective Remission

DISCUSSION

Luteotrophin induced the secretion of small amounts of milk in a group of women with mammary cancer despite the earlier removal of the ovaries The duration was impressive, as in all cases milk persisted for many months It was evident that neither adrenalectomy nor the administration of steroidal hormones were efficacious in suppressing lactation

Although the mechanism whereby this type of lactation is maintained for such long periods of time is not clearly understood, it seems certain that the anterior pituitary gland plays an important role in the initiation and maintenance of lactation since hypophysectomy in 3 patients caused cessation of lactation It is conceivable that luteotrophin injected into these patients functions as a stimulus acting directly on the breast tissue to initiate lactation in patients who otherwise will not lactate spontaneously The endogenous production of lactogenic hormone from the pituitary is the essential factor for the maintenance of the secretion in these women

The observations of objective regression of cancer following adrenalectomy in patients having no lactation after luteotrophin and the unfavorable

response to adrenalectomy in those who lactate after luteotrophin are indeed most interesting. The significance of these observations cannot be ascertained at this time. It is most unlikely that pituitary lactogenic hormone *is a growth promoting factor in breast cancer since the removal of the pituitary* gland in all 3 patients has failed to produce any regression of cancer although lactation has been successfully suppressed in all cases.

SUMMARY

Luteotrophin was given as a stimulus for mammary secretion to 11 women with metastatic breast cancer. Six patients lactated and 5 failed to lactate following the administration of luteotrophin.

Neither adrenalectomy nor steroid hormones were efficacious to suppress lactation.

Hypophysectomy caused cessation of lactation in all 3 patients who underwent the operation.

Luteotrophin failed to induce lactation in a castrated, adrenalectomized and hypophysectomized woman who had lactation before hypophysectomy.

The luteotrophin used in this study was generously supplied by E. R. Squibb & Sons and by Professor C. H. Li of University of California.

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STEROID METABOLISM OF THE HUMAN OVARY UNDER PERFUSION WITH HEMOGLOBIN SOLUTION*

GEORGE S. RICHARDSON, HOWARD ULFELDER, AND LASZLO VINCZE

Since the discovery by Baggett, *et al.*,[1] that human ovarian slices could convert testosterone to estradiol —17β, evidence has mounted to show that testosterone is probably the normal immediate precursor substance in estrogen synthesis.[2]

The present studies were initiated in the hope of providing improved and yet simple conditions for this and other reactions, so that human ovaries obtained at operation could be studied routinely by this means as well as histologically.

Knowing the limitations of slice preparations,[3] and the difficulty of homogenization of the ovary because of its dense collagen structure and the small fraction of it which at any time is hormonally active, we have worked on a perfusion technique. We have chosen to perfuse the ovaries through the arteries rather than in retrograde fashion through the veins, as has been done with the adrenal.[4] After a number of unsuccessful experiments

* From the Vincent Memorial Hospital and the Gynecological Service of the Massachusetts General Hospital, Boston. Supported by Research Grant C-4009 of the National Cancer Institute of the National Institutes of Health, Public Health Service.

using whole blood as a perfusion medium we have abandoned it for a variety of reasons most of which are long familiar to physiologists. 1) vasoconstrictor materials (serotonin, epinephrine) in whole blood, fresh or stored, may act unpredictably to make perfusion impossible, 2) even in the presence of an excess of anticoagulant, minute thromboses can occur which can be disastrous in small organ perfusion, 3) red cell agglutination with vessel obstruction may occur if there is not complete compatibility between blood and organ donors, 4) a minimum viscosity of the medium is of great importance to aid in the perfusion of as much of the tissue as possible for the longest possible time, 5) from the chemical viewpoint it is practically essential that the same medium be used in every experiment.

The use of a hemoglobin solution overcomes these difficulties and its properties have been shown to be well suited to circulatory studies by the perfusion technique.[5] The molecular size of hemoglobin approximates that of serum albumin so that with adjustment of electrolyte and glucose concentrations it is possible to provide a medium which has an oxygen-carrying capacity about half that of whole blood and a colloid osmotic pressure corresponding to that of plasma. Furthermore the material, which is relatively simple to manufacture in bulk, will remain stable with respect to oxygen carrying capacity for many months at ordinary ice box temperature (+5° C.). An unforeseen dividend has been due in part to the absence of lipid so that extracts have been clean and emulsion formation in the many solvent partition steps largely absent.

In the experiments reported below we have in some instances mixed the slice or homogenate method with that of perfusion in that we have suspended a homogenate of the ovaries in the perfusion fluid and extracted both together. We have also pooled the material from two perfusions (total of 4 ovaries) on two occasions and have worked them up together.

METHOD

Approximately 20 liters of a solution of human hemoglobin were prepared by the method of Hamilton, *et al.*,[6] in December, 1957. After the addition of one million units of crystalline penicillin this was Seitz filtered, bottled in 500 ml. units and stored at +5°C. This material has the following composition: sodium 110 mEq./L., chloride 102 mEq./L., potassium 1 mEq./L.; calcium 0 mEq./L., phosphorus 6 mEq./L., magnesium 2.9 mEq./L., glucose 19 mg %, total nitrogen 10.51 mg %, nonprotein nitrogen 4.00 mg %, total protein 6.56 gm %, photoelectric hemoglobin 5.5 gm % (photoelectric), active hemoglobin (Van Slyke) June 17, 1958, 5.2 gm %. Immediately before use calculated small amounts of concentrated solutions of sodium bicarbonate potassium chloride and calcium chloride were added as was also glucose. For example, in Case 2 reported below the sodium concentration was 136 mEq./L., chloride 91 mEq./L., potassium 4.0 mEq./L., and the pH (Beckman meter) 7.5. No attempt was made to provide gonadotropin or cofactors. The solution was oxygenated with a mixture of 95% oxygen—5% carbon dioxide using an improvised disc oxygenator. In experiments in which a small volume (250 ml.) of hemoglobin solution containing radioactivity was recycled through the ovary, reoxygenation was carried out simply by bubbling 95% oxygen—5% carbon dioxide into the fluid.

Human ovaries resected in such a manner as to preserve a long pedicle

without crushing were obtained in the operating room, flushed at once through their vessels with heparin in cold saline, and placed on ice

The ovarian arteries were cannulated and flushed with prepared cold hemoglobin as soon as the glands reached the laboratory The vessels are small the veins usually being larger than the arteries but thinner walled, and usually not more than three of these vessels per ovary can be cannulated There was thus always some collateral arterial leakage Perfusion was then carried out by gravity in the simple manner illustrated for a period of about 6 hours Testosterone —4—C^{14}, obtained from New England Nuclear Corporation, specific activity 4 13 mc /mg or about 5700 counts/minute/μg was dissolved in 1 ml of 95% ethanol, mixed with 10 ml of the hemoglobin solution, and then added to the perfusion fluid or injected into the tubing The glands and perfusate if not immediately extracted were stored at —20° C The chemical methods used are those described by Engel, *et al* [7] For extraction, we are grateful to Mr Robert Purdy for the use of an unpublished method

RESULTS

Injection Studies. Initially, a number of studies of the human ovarian vasculature using latex rubber injection and microscopic section were carried out to test the accuracy of arterial cannulation We now feel that the arteries can be recognized and selected with virtual certainty As a check on the completeness of perfusion, india ink has been added to the hemoglobin after a few hours of perfusion In most instances complete perfusion of the parenchyma has been demonstrated The photomicrograph is from Case 2 and shows the preservation of a normal histology and the presence of hemoglobin rather than red cells in both arteries and veins

Tissue Survival Studies. In order to test the duration of tissue viability we have followed A V oxygen differences of similarly perfused rabbit kidneys Although the kidney is not a very satisfactory organ for perfusion perhaps because of its rather special vasculature, we selected it because of its availability, size comparable to the human ovary and the presence of a single artery and vein Flows were very slow (average —0 05 ml /gm /min) but quite constant, and although by india ink injection medullary perfusion was good at the end there were spotty unperfused areas in the cortex In 13 determinations in 3 experiments the oxygen carrying capacity of the "venous hemoglobin averaged 6 5 vol % (range 5 4 to 7 0), the oxygen



Fig 1 Low power photomicrograph (80 ×) of ovary from Case 4 after 6 hours of perfusion Note absence of red cells from vessels

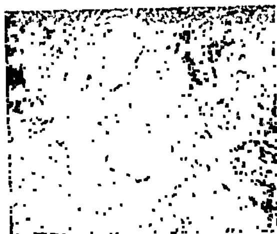


Fig 2 Same as Fig 1, high power (250 ×) Note appearance of hemoglobin in vessels and normal histology without edema

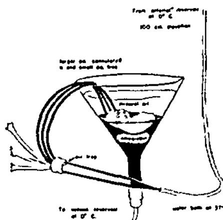


Fig 3 The perfusion setup semidiagramatic hemoglobin is kept active by refrigeration except for warming just before entry into the ovary Catheters are polyethylene (PE 60 and PE 90) Ends are heated and crushed before use, being cut as needed since the number of cannulatable vessels is variable The ovary floats at the oil water interface The apparatus is in a water bath at 37°C (stippled area)

content averaged 3 1 vol % (range 1.4—5 0), or an average difference of 3 4 vol % In the first experiment, the difference determined at 6 hours after start of perfusion was 4 9 vol % and in the second and third at 4 hours it was 3 1 and 3 3 vol % respectively In the ovarian perfusions, however, there was too much "arterial" admixture for there to be a significant A-V difference

Ovarian Perfusions. Six perfusions with radioactive testosterone have been carried out Three of these present the problem of conversion to unidentified material as illustrated by Case 1 below Unfortunately this material is different in each instance The other two were menopausal ovaries showing cortical stromal hyperplasia

Case 1 (MGH 970158) The ovaries were obtained from a 21 year old woman undergoing total hysterectomy for an adenoacanthoma of the endometrium present for one year The patient had been bleeding irregularly, but the ovaries were

(about 1500 ml) was freeze dried and extracted Figure 4 shows the results of a 105 transfer countercurrent distribution The solid curves are theoretical for the partition coefficient (K) and peak tube values indicated E_1, E_2 and E_3 fluorescence values are those of the estrone, estradiol and estriol added as carrier immediately prior to the distribution A resin column was used in the workup to separate neutral from phenolic steriod, the 'phenolic' fraction only was placed in the distribution

The radioactivity is seen as a high background level with a single peak in an unexpected position This is not that of testosterone, which should be almost super imposed on the estrone curve, yet it has proved to be neutral by means of a sub sequent toluene normal sodium hydroxide distribution and consequently is not any kind of estrogen There is no more of this compound available for study and more experiments will be needed to show whether it will occur as a metabolite of testosterone

Cases 2 and 3 (MGH 1010875 and 998026) Here again both ovaries of two

without crushing were obtained in the operating room flushed at once through their vessels with heparin in cold saline and placed on ice

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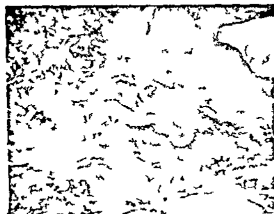


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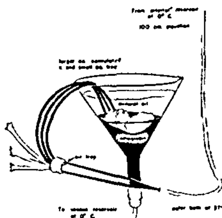


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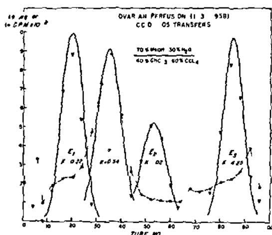


Fig 4 Case 1 *See text*

patients were perfused with recycling Both patients had carcinoma of the breast In the first patient, aged 44, the ovaries showed a corpus luteum and the endometrium was postmenstrual, in the second aged 32, the ovaries showed corpora lutea and a follicle cyst and the endometrium was secretory A total of 1 449 000 counts per minute of testostrone 4 C^{14} per microgram was used in both perfusions The hemoglobin solution and the ovaries were extracted together The 100 transfer distribution is shown in Figure 5 Only about 500 000 counts of the original testosterone remains from the previous steps to appear in this distribution, but about 60 000 counts of estradiol has appeared i e about 10% of the recovered radioactivity is estradiol Although no estradiol carrier has been added and there is much nonspecific fluorescence and background fluorescence, a small but definite estradiol peak is present The specific activity of this estradiol is roughly 3400 counts/min /µg as compared to 5700 counts/min /µg for the original testosterone By this admittedly crude measure a surprisingly large part (60%) of the estradiol present has been newly manufactured from testosterone Since the glands themselves were also extracted one might have expected more preexisting estradiol That the material obtained is in fact estradiol has been demonstrated by the subsequent distribution after addition of carrier shown in the final graph (Figure 6) This has been submitted to statistical analysis for radiochemical purity by the method described by Baggett and Engel [8] The specific activity is constant in the peak area with a coefficient of variation of 12% Estrone and estriol have been sought for in this experiment but not found

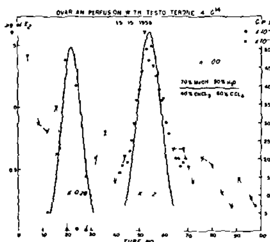


Fig 5 Cases 2 and 3 Estradiol peak (K equals 1.2) is shown at 10 × magnification in comparison to testosterone peak (K equals 0.28)

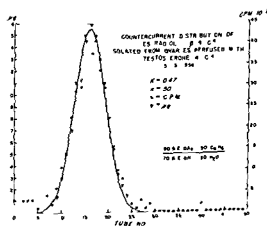


Fig 6 Cases 2 and 3 Redistribution in another system of the estradiol zone of Figure 5 after addition of pure estradiol carrier

SUMMARY

1 A simple method of ovarian perfusion is described

2 This method is shown to be serviceable for steroid studies involving biochemical interconversions

3 In three experiments involving five perfusions in which radioactive testosterone —4—C^{14} was used its conversion to estradiol —17β was demonstrated only in one experiment in which both pairs of ovaries showed active corpora lutea Estrone and estriol were sought for but not found

4 Suggestive evidence of conversion of testosterone to other unidentified compounds is also presented

The authors wish to thank Dr L L Engel of the Huntington Memorial Laboratories of the Massachusetts General Hospital for advice and the loan of laboratory facilities Dr R B Pennell of the Protein Foundation Jamaica Plain Massachusetts for help in the bulk preparation of hemoglobin and the State Laboratories Commonwealth of Massachusetts for bottling and sterility testing of the material

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ELECTROLYTE BALANCE STUDIES WITH A NEW ALDOSTERONE ANTAGONIST IN THE TOXEMIAS OF PREGNANCY*

JOHN C BUCKINGHAM AND ALLAN C BARNES

In recent years the electrolyte changes incident to the eclamptogenic toxemias have been documented with increasing clarity Less clear has been the answer to the question are these metabolic disturbances the result of the toxemic process or are they etiologically related?

The introduction of steroids which have antialdosterone properties provides an avenue of approach to this question Previous studies by the authors and others [1, 2, 3, 4] indicate that this chemical SC 8109 G D Searle and Company acts as an effective antialdosterone agent when that steroid is

* From the Department of Obstetrics and Gynecology Western Reserve University School of Medicine Cleveland Aided by a grant from the Cleveland Area Heart Society

present although in the absence of aldosterone its impact on the electrolyte status of the patient is not necessarily the opposite of the effect of aldosterone

Aldosterone has been shown to be excreted consistently by the toxemic pregnant woman [5] and less consistently by many nontoxemic women in the last trimester [6] The present study evaluates the effect on preeclampsia of the administration of the antialdosterone SC 8109

METHOD

The women being studied were admitted to a balance ward for the careful measurement of all intake and output of fluids and electrolytes Sodium and potassium were measured by flame photometry chloride by Sendroy modified by Van Slyke and Hiller nitrogen by macro Kjeldahl No attempt was made to extract aldosterone chromatographically but the sodium retaining activity of the chloroform extract of selected patient s urine was assayed in adrenalectomized rats [6,7,8]

Eleven women were studied These included 2 normal pregnant and one normal cesarean hysterectomy patient none of whom received the medication but who were followed during the early puerperium for electrolyte shifts associated with the early postpartum changes One preeclamptic pregnant patient was studied without medications

SC 8109 was administered to one nonpregnant woman to 2 with normal pregnancies and to 4 women with preeclampsia of varying degrees of severity A total of 123 patient days in the balance ward serves as the basis for this report

RESULTS

The normal controls who received no medication amply confirmed the pregnant patient s tendency to retain sodium and to release this in quantity during the immediate puerperium Measurements of aldosterone levels were not carried out in these particular women although previous studies in this clinic have indicated that while many nontoxemic pregnant women excrete aldosterone in their urine this sodium retaining tendency is not necessarily in proportion to the amount of this steroid thus elaborated Undoubtedly there are other factors contributing to the sodium and water retention of the last trimester of pregnancy

The balance study of the nonpregnant control patient who received SC 8109 is illustrated in Figure 1 This 33 year old unmarried woman complained of intermittent spontaneous episodes of water retention and weight gain and stated that she was salt sensitive It can be seen that the administration of the steroid being studied resulted in the increased liberation of sodium with some degree of diuresis Assays of the sodium retaining activity in the chloroform extract of her urine however did not coincide completely with the clinical response as indicated by the alleviation of her fluid retention She does not on laboratory investigation fit the syndrome of hyperaldosteronism although the oral form of SC 8109 keeps her subjectively more comfortable than any other therapeutic program thus far attempted

One of the normal pregnant controls is illustrated in Figure 2 It can be seen that after a period of time achieving balance zero there is a prompt release of sodium and fluid in response to the administration of this steroid This was characteristic of both of these control women although there were no clinical signs of toxemia in either case Once again the assay in the

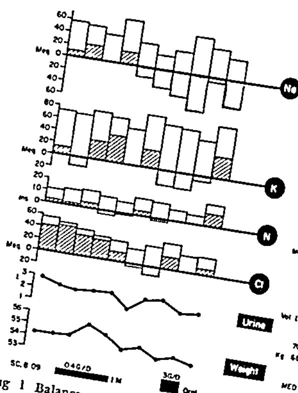


Fig 1 Balance studies on a nonpregnant control Intake measurements are recorded from the baseline upwards in the units indicated Output measurements for any given day are then superimposed starting from the top of the intake column downwards When the output exceeds the intake, the excess (below the baseline) is shaded, when the output is less than the intake, the difference (above the line) is indicated with cross hatching It can be seen in this case that the antialdosterone produced a marked increase in the elimination of sodium, although there was no marked associated diuresis

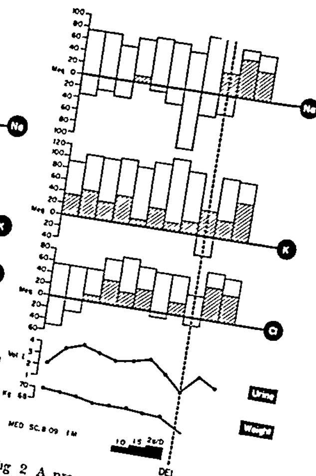


Fig 2 A pregnant nontoxemic control patient. As in the case of the patient illustrated in Figure 1, the administration of SC-8109 led to marked elimination of sodium

adrenalectomized rat, of the sodium retaining factor in the urine (carried out in one of the control patients) did not show good correlation with the renal response as far as the electrolyte balance and fluid elimination were concerned

Looking at this balance study, however, one would be encouraged with respect to the usefulness of this drug in the eclamptogenic toxemias In particular, the sodium response which is demonstrated here is the desideratum of our therapy in combatting the electrolyte imbalance of the toxemias of pregnancy

Figure 3 illustrates, however, the response which was observed in all 4 of the women with toxemia who were studied There is no good evidence in this study that there was a specific reversal of the underlying metabolic

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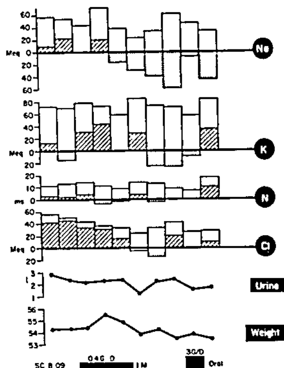


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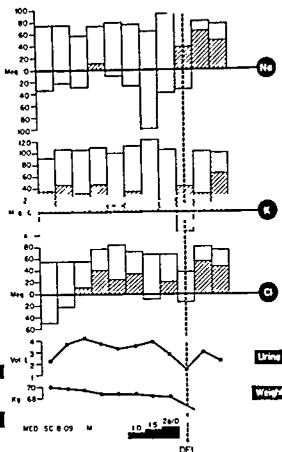


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Figure 3 illustrates, however, the response which was observed in all 4 of the women with toxemia who were studied There is no good evidence in this study that there was a specific reversal of the underlying metabolic

derangement, nor was there a reversal of the clinical syndrome in any of these patients

The toxicity to SC 8109 was manifest in these patients most consistently in the form of nausea. This was mild, passing, and seldom interfered with completing the course of study. In one patient somnolence and some confusion were noted, which quickly reversed on discontinuing the medication.

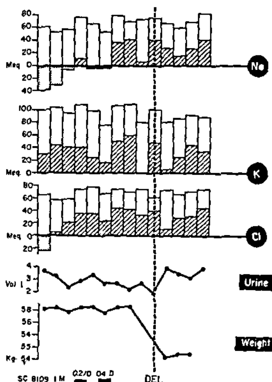


Fig 3 Balance study in a patient with marked preeclampsia. In this case, as with other toxemic patients, the intramuscular administration of SC 8109 was not associated with a comparable elimination of sodium

DISCUSSION

In essence these findings indicate that the action of this drug, SC 8109, to eliminate retained sodium in pregnancy is most marked in those cases where it is therapeutically least needed. The effectiveness in the toxemias is minimal, where the need for sodium elimination is greatest, whereas its chief demonstrable effect is in the nontoxemic group. This observation is not necessarily in conflict with the conclusion of Salassa[4] that SC 8109 is an aldosterone antagonist when that steroid is present, but does not possess opposite electrolyte effects when aldosterone is not present, since the excretion of aldosterone in pregnant patients is known not to be necessarily proportionate to any signs of toxemia

The difference in observed effectiveness between the normal pregnant and the preeclamptic women could represent the need for considerably higher doses in the latter group, or it could simply be a fundamental difference in kidney response which occurs as part of the chain of events in the toxemia process

CONCLUSION

1 A series of metabolic balance studies have been reported in pregnant women with and without the eclamptogenic toxemias, fundamentally measuring the impact of a new aldosterone antagonist (SC-8109) on the electrolyte and water metabolism

2 In the women tested, this drug reversed the sodium retaining tendency

observed in normal pregnancy but was without the corresponding electrolyte effect in the presence of preeclampsia. The clinical signs and symptoms of toxemia were not significantly effected by the administration of this antagonist.

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THE EFFECT OF ORAL ESTROGENS UPON THE IMMEDIATELY POSTPARTUM ENDOMETRIUM AND VAGINAL MUCOSA*

C A Douglas Ringrose

Numerous reports have appeared describing the efficacy of various estrogens for the suppression of lactation in the postpartum period. Side effects such as subinvolution and withdrawal bleeding have been reported following such therapy. Most of these reports have been lacking in objective criteria and lean heavily on the impressions of the patient which are undoubtedly further modified by the interpretation of the doctor. A notable exception was the report of Gross *et al*. Using the double blind technique they examined the effect of various hormone regimen and placebos on postpartum pain, engorgement and lactation. They found the placebo almost equal to the most effective hormonal regime in the relief of pain (87% vs 93%). The second part of their study dealt with the appearance of the endometrium in the above described groups during the periods 5 to 7 days and 21 to 30 days postpartum. In addition several observations at other times were described. They recognized no difference in endometrial characteristics between nursing and nonnursing mothers until the 21 to 30 day period. Moreover no effect of Vallestril was demonstrable on the endometrium in either nursing or nonnursing groups. They state that conjugated estrogens which were no better than the placebo for suppressing pain and lactation may accelerate epithelial regeneration. Methyltestosterone was found to have no noticeable effect. Rutherford in 1942 stated that Stilbestrol was responsible for increased mitotic activity and complete epithelial regeneration by the 8th to 9th day. He felt that stromal regeneration however was slowed by Stilbestrol. It is apparent then that the postpartum endometrium is relatively refractory to the influence of hormones in the dosages usually employed.

To ascertain the degree of refractoriness the author administered various

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* From Herman Kiefer Hospital and the Wayne State University College of Medicine

dosages of estrogens to a group of 15 nonnursing mothers Vaginal smears were obtained from the lateral wall in the upper third at various times from the 3rd to the 14th postpartum day and stained by the Papanicolaou technique Cervical smears were also taken Endometrial biopsies were secured in all cases on the 14th postpartum day and at various others times Two control groups (10 subjects each) receiving no hormones were examined in the same manner One group of 10 was breast feeding and the other was not

In 50% of the control groups, no hormonal reading of the vaginal smear could be made because of Trichomonas infestation Papanicolaou and Wolinska have called attention to the acidophilia and cornification that Trichomonads produce in the vaginal smear Results in the remainder indicate that the typical pregnancy smear type with its folding and crowding of intermediate and superficial noncornified cells, starts to manifest basal cells on the 3rd or 4th postpartum day The percentage of atrophic cells continues to increase, particularly in the nursing mothers, until on the 14th postpartum day some smears showed as high as 80% parabasal and basal cells (Fig 1) The range was great, however, and others showed as little as 20% atrophic cells with the balance being intermediate cells In the nonnursing group receiving no hormones, one third of the cases showed no atrophic cells and began to display increasing numbers of superficial cornified cells from the 14th day onward The cervical smears were generally "cluttered" with leukocytes, red cells and mucus (Fig 2) Glandular cells were seen frequently (Fig 3) Usually they were swollen and chromatolytic when compared to the "donor" glands in the biopsy specimens

The endometrial biopsies were secured from at least two areas to rule out sampling of nontypical islands The epithelium of the control groups at 2 weeks was cuboidal and in some areas flat The nuclei of the epithelial cells frequently were chromatolytic, the nuclear membrane alone taking the stain (Fig 4) This was especially marked in the breast feeding group The stroma showed some infiltration with inflammatory cells Inflammatory cells were also seen in the remnants of hyalinized decidua being undermined by epithelial regeneration (Fig 5) The stromal cells in some areas appeared



Fig 1 Atrophic vaginal smear from lactating woman on 14th postpartum day

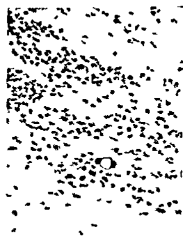


Fig 2 Cervical smear displaying basal cells leukocytes and degenerating red cells on 14th postpartum day from nonnursing mother not treated with Stilbestrol



Fig 3 Glandular cells in Vaginal smear on 14th postpartum day



Fig 4 Endometrium on 14th postpartum day in nursing mother Note chromatolytic nuclei of epithelial cells

to be "surfacing" to assume an epithelial shape and function (Fig 6) This 'metaplasia undoubtedly supplements regeneration from basal glands as well as upgrowth from the endocervix and perhaps down growth from the tubal mucosa



Fig 5 Endometrial decidual junction in nonnursing nonhormone treated mother on 14th postpartum day Note similarity between stromal nuclei and epithelial nuclei



Fig 6 "Surfacing" cells in nonlactating mother on 3rd postpartum day Subject has received 75 mg of Stilbestrol

In the hormone treated group, large doses of Stilbestrol (25 mg daily), administered for 2 weeks produced a moderate to marked proliferative effect on the endometrium (Fig 7) The stroma was clean and succulent The glands became numerous and were lined with columnar epithelium The nuclei were normochromic Subnuclear vacuoles were numerous That the vacuoles are a characteristic of intense estrogen effect as stated by Hisaw, and not a criterion of ovulation was confirmed by followup biopsy one week after cessation of hormone therapy This subsequent biopsy *showed no* evidence of secretory effect Time was necessary for marked proliferation to occur and 12 to 14 days appeared to be the minimal period for maximal change Dosages as high as 50 mg of Stilbestrol b i d produced no greater effect than 25 mg daily in 7 days

The vaginal smear was a much earlier indicator of estrogenic effect By the 3rd or 4th day of therapy *all the cells were of the* superficial type with about one third cornified At the end of the 2 week period 95% of cells were cornified and lying flat and singly (Fig 8) The smears were very "clean" compared to the controls, with few leukocytes and red cells The flora showed

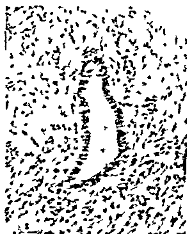


Fig 7 Endometrium from hormone treated (350 mg Stilbestrol) nonnursing mother on 14th postpartum day Note abundant clean stroma columnar gland cells with normo chromic nuclei and subnuclear vacuolation

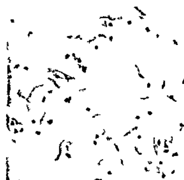


Fig 8 Vaginal smear in hormone treated woman on 14th postpartum day displaying cornified cells lying flat and singly

B vaginalis commonly when the hormonal reading could be made Here again, *Trichomonas* infestation made the reading impossible in about 50% of cases

Side effects were few One subject displayed marked pitting edema at the end of the two week period presumably due to the water retaining effect of the steroid Another subject reported a tremendous upsurge of libido while taking the Stilbestrol This was probably coincidental, and returning home to an amorous husband was undoubtedly a great factor in this symptom In none of 15 hormone treated patients did serious withdrawal bleeding develop Usually there was a period of 4 to 6 days after therapy was stopped before withdrawal bleeding occurred This lasted 3 to 4 days and simulated a light period In 2 cases spotting for 10 days and 8 days respectively occurred after cessation of therapy No rebound filling of breasts occurred following therapy and no 'subinvolution was noted at the end of 6 weeks

Two further cases are noteworthy Both developed a postpartum endo metritis One case was receiving no hormones and was not breast feeding Uterine culture yielded a micrococcus which was not a *Staphyloccus* Antibiotic therapy was effective in relieving all sign and symptoms However endometrial biopsy at the end of 2 weeks was accompanied by the exit of frank pus from the cervical os The patient at this time was asymptomatic and showed no other signs of local or systemic infection The biopsy showed



Fig 9 Endometrium on 14th postpartum day from woman clinically cured of endo metritis 5 days previously Subject received no hormones and was not breast feeding

a stroma infiltrated with inflammatory cells. The glands were cuboidal with chromatolytic nuclei and the lumina contained pus (Fig. 9).

The other case also developed signs and symptoms of endometritis and culture yielded diphtheroids, paracolon and *E. Coli*. Stilbestrol 25 mg. daily for 2 weeks was added to the usual antibiotics. The 2 week biopsy in this case showed a fairly abundant clean stroma. Glands were numerous and low to midcolumnar in character. Nuclei were chromatin normal.

SUMMARY AND CONCLUSIONS

Lactation in the majority of cases produces atrophic changes in the vaginal epithelium and uterine endometrium. In the vaginal smear this is characterized by an increase in the precentage of basal cells. In the endometrium the stroma is scanty and hyalinized decidua is prominent. The epithelium is low cuboidal and the gland cell nuclei are chromatolytic.

Dosages of estrogens usually employed for the suppression of lactation have little if any effect on the endometrium in the first 2 to 3 weeks. There is, however, a somewhat greater proliferative effect on the vaginal epithelium. This is in keeping with the observation of Wied and others that the sensitivity of the vaginal epithelium to hormones is 5 times greater than that of the endometrium.

Large dosages of Stilbestrol are capable of overcoming the *refractoriness* of the genital tract lining. No serious adverse effects were noted.

It is suggested that the economical anabolic effect of Stilbestrol in the large dosages employed may be a useful addition to the usual measures for handling endometritis.

This study tends to support the concepts that stromal metaplasia can occur and that subnuclear vacuoles in uterine epithelium are indicative of intense estrogen effect.

MELANOMA AND PREGNANCY *
AN EXPERIMENTAL EVALUATION OF A CLINICAL IMPRESSION

Everett C. Shockey and Joseph G. Fortner

The development and spread of a melanoma is generally believed to be exacerbated in the patient who also has a concurrent or recent pregnancy. This impression is based largely on information obtained from scattered single case histories[1, 2, 3, 4, 5] and on a small uncontrolled series of patients.[6] *In these reports* the dramatic exacerbation of a melanoma growth is related to the pregnancy of the patient. The significance of such reports is vitiated by their

* From the [illegible]
Kettering In[illegible]
Memorial C[illegible]
[illegible] the National Institutes of Health, U. S. Public Health Service. With the technical assistance of Miss Alice Gale.

sparsity and by the occurrence of similar dramatic exacerbations in the course of nonpregnant or of male melanoma patients

Melanoma growth is probably influenced in some as yet undefined way by the endocrine status of the host Pigmentary changes normally accompany pregnancy but further study is necessary to establish that growth of the melanoma cell is also affected The experiment herein recorded was designed and carried out to provide more information on this problem

METHOD

The experimental model was the Syrian Golden hamster bearing a transplantable melanotic melanoma This melanoma (designated as H M M #2) arose spontaneously in the Syrian Hamster [7, 8] and is one of 7 which have been established in transplantable form in our laboratory It is particularly suited for this type of experiment since its gross microscopic and biologic features seem to simulate those of many melanomas in humans Furthermore its rates of growth and of metastatic spread are such that alterations are detectable within the limits imposed by the hamster s gestation time plus a short period of lactation Thus the melanoma reaches a size of approximately 4 $\times$ 2 5 cm in 30 days It metastasizes to ipselateral axillary or inguinal nodes in one sixth of implanted animals during the first 50 days of transplant growth Pulmonary and hepatic metastases are seen after the transplanted tumor has been *in situ* for about 100 days

The Syrian Golden hamsters of this experiment were young adults of homogeneous appearance which had stabilized in the laboratory They were obtained as weanlings from a commercial dealer The hamsters were maintained throughout the experiment on Purina Laboratory Chow with carrots and oats as a supplement and water *ad libitum*

Upon initiation of the experiment 49 female hamsters were each inoculated with 0 5 cc of a fine suspension of H M M #2 melanoma The suspension was prepared by mincing 15 gm of tumor in 30 cc of sterile physiological saline The mince was then passed through a #40 wire mesh Cell counts of representative samples revealed that 0 5 cc of the suspension contained about 63 million cells The injections were made subcutaneously in the right flank with a 1 0 cc tuberculin syringe through a 16 gauge needle

Forty eight hours after implantation of the tumor the animals were grouped so that 2 inoculated females were housed with a mature uninoculated male After 5 days each female was individually caged and observed (the hamster s estrus cycle is of 4 days duration)

The 15 hamsters which became pregnant bore litters and were permitted to nurse their young These animals constitute the test or pregnancy lactation group The remaining 34 inoculated female hamsters which failed to become pregnant constitute the control or nonpregnant group Both test and control groups were sacrificed in parallel 29 to 32 days after tumor transplantation This was 10 to 12 days after parturition for the pregnancy lactation group

Each animal was carefully autopsied The tumefaction at the inoculation site was inspected removed and weighed The tumor free animal carcass was weighed and a detailed search made for gross metastases Regional and distal nodes as well as representative sections of viscera were studied microscopically

RESULTS

The weights of the tumor transplants are shown in Table 1 and in Figure 1. These demonstrate that the mean weight of melanomas in animals of the pregnancy-lactation group is less (7.97 gm.) than that of the control or non-pregnant group (10.48 gm.). Subjected to analysis of variances, this difference is found to be significant at the 5% level.

Animals of the pregnancy-lactation group were generally somewhat smaller than their controls at time of sacrifice (Table 2). The difference is not statistically significant. Furthermore, there is no correlation of tumor weight with tumorless carcass weight as shown in the scatterdiagram of Figure 2.

No grossly discernible metastases were present. Microscopically, however, small foci of metastatic melanoma were found in both the test and control groups. Regional lymph nodes were involved in 3 of 15 animals of the pregnancy lactation group and in 3 of 17, randomly selected, animals of the

Table 1. Melanoma and Pregnancy

GROUP	NO	WEIGHT OF TUMOR (GM)		
		RANGE	MEAN	STANDARD DEVIATION
Pregnancy—Lactation	15	3 08 13.23	7 97	2 76
Control	34	5 94 19 73	10 48	2.88

Fig 1

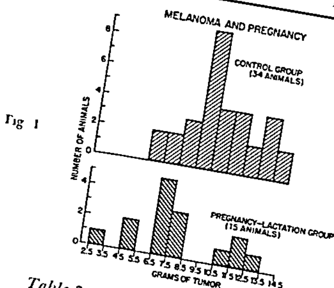


Table 2 Melanoma and Pregnancy

GROUP	NO	WT OF TUMORLESS CARCASS (GM)		
		RANGE	MEAN	STANDARD DEVIATION
Pregnancy—Lactation	15	82 1 112.2	90 6	7 32
Control	34	81 7 111 8	95 1	8.55

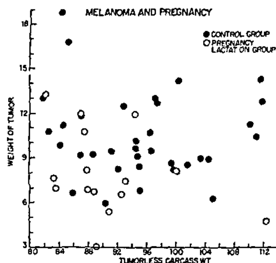


Fig 2

control group (Table 3) Visceral metastases were not found There appeared to be no difference in the intensity of pigmentation between the melanomas in the two groups

Table 3. Melanoma and Pregnancy

	PREGNANCY-LACTATION GROUP	CONTROL GROUP
Gross Findings		
Number examined	15	31
Metastases	0	0
Microscopic Findings		
Number examined	15	17
Metastases †	3	3

† Metastases when present limited to microscopic foci in regional nodes

DISCUSSION

The data of this experiment clearly indicate that under the conditions described, pregnancy and lactation of the host did not stimulate either the local development or the metastatic spread of a transplantable hamster melanoma (H M M #2) Rather, growth of the tumor as measured by its weight appears inhibited No effect was exerted on the tumor's ability to metastasize

The apparent suppression of local tumor growth by pregnancy and lactation of the host was an unexpected finding Selection of individual animals for the test and control groups was based on the happenstance of pregnancy This raises the possibility of a selective process However, the incidence of pregnancy was that expected using the inefficient mating procedures of this experiment It seems unlikely that animals of the two groups initially differed in any significant way

Some of the "pregnant lactating animals weighed less at time of sacrifice than the controls (Table 2) This is reasonably attributable to the effects of pregnancy and of nursing a litter Relatively smaller tumors in test animals cannot be ascribed simply to a smaller host size for the scatterdiagram of

that there is no correlation between tumor weight and host

ce in tumor mass of the test and control groups is of a signifi le It appears related only to pregnancy and lactation of the

CONCLUSION

The effects of pregnancy and lactation on the local growth of a transplanted hamster melanoma (H M M #2) and on its propensity for metastatic spread has been studied Under the experimental conditions growth of the mela noma implant appears to have been inhibited The tumor s ability to metastasize was unaffected

The authors are greatly indebted to Drs S S Sternberg Irwin J Bross H T Randall and I S Ravdin for their invaluable assistance and support

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WATER EQUILIBRATION BETWEEN THE FETUS AND THE MATERNAL ORGANISM IN HUMANS AT TERM *

HARRY S MCGAUGHEY JR WILLIAM A SCOGGIN
HERBERT C JONES JR W NORMAN THORNTON JR AND
W PARKER ANSLOW JR

In a previous study evidence was adduced for the concept that water is in osmotic equilibrium at the placenta [1] In the present report the osmotic pressures were determined in maternal blood (arterial venous and intervil lous samples) in fetal blood (umbilical artery and vein samples) and in amniotic fluid Analysis of the data confirms the supposition of osmotic equilibration of water at the placenta and suggests that this equilibration transfers free water † from maternal to fetal circulations A cycle is proposed in which the free water is secreted from the fetus and returned to the maternal body by absorption at the amniochorionic membrane

† The term free water signifies water unaccompanied by solutes and was introduced by renal physiologists to describe the dilution of the urine in water diuresis and the con centration of the urine in antidiuresis

* From the Departments of Obstetrics and Gynecology and of Physiology University of Virginia School of Medicine Charlottesville Supported in part by research grant H 2620 (C1) from the National Heart Institute Public Health Service

METHOD

Studies were carried out on patients, at term, delivering vaginally Maternal intervillous blood was obtained by the technique of transabdominal aspiration [1] Fetal arterialized blood was obtained from the anatomic umbilical vein and fetal venous from the anatomic umbilical artery, both just prior to cord ligation Samples of amniotic fluid were obtained either by transabdominal aspiration or by amniotomy Maternal samples were taken from the brachial artery and antecubital vein Following centrifugation, the total solute concentration of the various samples was determined in a modified Fiske Osmometer

RESULTS AND DISCUSSION

To eliminate the variability of solute concentration from individual to individual a series of ratios were studied Four of these ratios (C_{PS}/C_{MV}, C_{FA}/C_{MV}, C_{MA}/C_{MV}, and C_{AF}/C_{MV}) compare the solute concentration in placental sinus plasma, fetal arterialized plasma maternal arterial plasma and amniotic fluid supernatant, respectively, to solute concentration in maternal peripheral venous plasma C_{FA}/C_{PS} compares the concentration of solute in fetal arterialized to that in placental sinus plasma, and C_{FV}/C_{FA} compares the solute concentration in fetal venous to that in fetal arterialized plasma †

Table 1 collates data for the 6 ratios, including means, standard deviations and significance of the deviation of each mean value from 1 00 ('T value)

The mean value for C_{FA}/C_{PS} is 0 998, and its 'T' value indicates that statistically it may be considered 1 00 Since a ratio of 1 00 signifies that both samples have the same osmotic pressure, fetal blood appears to be in osmotic equilibrium across the placental barrier with maternal blood, thus confirming directly our earlier supposition

Inspection of Table 1 shows that C_{PS}/C_{MV} has a mean value of 1 013 which is significantly greater than 1 00 This circumstance suggests either that maternal blood loses water at the placental site, or that the ratio is physiologically spurious, since events in the placental circulation may not bear any relationship to those in the systemic However, C_{MA}/C_{MV} is not statistically different from 1 00 (Table 1) and therefore, substitution of C_{MA} for C_{MV} in all ratios where the latter is the denominator would yield ratios of equal significance with respect to arterial blood This circumstance suggests that free water is lost from maternal blood at the placental site

Loss of free water from maternal blood should be reflected in a change in solute concentration of fetal blood passing through the cord Reference to Table 1 shows that the concentration of solute in fetal blood entering the placental site in the anatomic umbilical artery is greater than that leaving the placental site in the anatomic umbilical vein Thus events on the fetal side of the placental membrane are in agreement with those on the maternal side

The facts that C_{FV}/C_{FA} is statistically greater than 1 00 and that $C_{FA}/C_{PS}=$ 1 00 (Table 1) suggest that placental transfer of free water is a consequence of the loss of a significant quantity of solute poor water from the fetus

† In symbolizing fetal plasma samples we have elected a physiologic rather than an anatomic designation Thus, C_{FA} refers to concentration in arterialized plasma obtained from the anatomic umbilical vein and C_{FV} refers to that in plasma from the anatomic umbilical artery

Table 1

RATIO	NO. DETERMINATIONS	MEAN VALUE	STANDARD DEVIATION FROM MEAN †	"T" VALUE †† (DEVIATION FROM 1.00)	SIGNIFICANCE OF "T" VALUE †††
C_{FS}/C_{MV}	59	1.013	0.021	1.75	HS
C_{FA}/C_{MV}	83	1.014	0.017	7.50	HS
C_{MA}/C_{MV}	40	1.001	0.010	0.63	NS
C_{AF}/C_{MV}	35	0.903	0.050	11.50	HS
C_{FV}/C_{FA}	47	1.011	0.020	3.77	HS
C_{FA}/C_{FS}	59	0.998	0.018	0.85	NS

$C_{MV} = 281 \pm 8$ mOsm/Liter (105 determinations)

† Standard deviation from mean $= \sqrt{\frac{\Sigma d^2}{n-1}}$

†† "T" value $= \frac{(X - M)\sqrt{N}}{\sqrt{\frac{\Sigma d^2}{n-1}}}$

††† HS signifies that the difference between the ratio and 1.00 has less than one per cent opportunity of being due to chance operating alone. NS signifies that the ratio does not differ significantly from 1.00.

Support for the latter concept is seen in the composition of amniotic fluid which is distinctly hypotonic to both maternal and fetal fluids a circumstance originally reported by Zangemeister and Meissl[2] and confirmed by Makepeace Fremont Smith Dailey and Carroll[3] In our studies C_{AF}/C_{MV} averaged 0 90 (Table 1) Although the origin of amniotic fluid remains debatable few secretions can result in the loss of free water from the fetal organism and thereby contribute to the hypotonicity of the amniotic fluid Urine and sweat are noteworthy examples of such hypotonic secretions

Since the neonatal kidney elaborates a urine hypotonic to both maternal and fetal plasma[2 3 4 5] it does not seem unreasonable that micturition is one route whereby the fetal organism may lose free water The paucity of amniotic fluid in renal agenesis[6 7] suggests that fetal micturition plays a prominent role in the formation of amniotic fluid It is doubtful however that micturition is the sole explanation of the free water loss from the fetus since estimates in the newborn indicate a glomerular filtration rate of from 5 to 20 ml /min[8 9 10]

The adult sweating apparatus is capable of maximal secretion at the rate of 4 000 ml /hr[11] and moreover the sweat becomes more hypotonic on prolongation of thermal stress[12] Carrying forward this analogy an adult exposed to an ambient temperature of 37°C for any significant interval would secrete copious quantities of sweat with a distinctly hypotonic composition Although little is known concerning fetal sweating *in utero* its potential as a source of hypotonic amniotic fluid must be assessed

To complete the cyle of water exchange between maternal and fetal organisms reabsorption of free water by maternal fluids from amniotic fluid must occur In view of the relatively large surface area of the amniochorionic membrane and the osmotic gradient between amniotic and maternal fluids such a possibility does not seem remote *In vitro* studies in this laboratory using a chamber system indicate that the amniochorionic membrane can equilibrate urea at a rate sufficiently rapid to account for a reasonable rate of nitrogen excretion by the fetus Assuming that urea follows water across these membranes the data suggest that water can be readily equilibrated across the amniochorionic membrane

CONCLUSIONS

1 Studies of solute concentrations indicate a decreasing order as follows fetal venous plasma (anatomic artery)>fetal arterialized plasma (anatomic vein)=maternal intervillous plasma>maternal venous plasma=maternal arterial plasma>amniotic fluid

2 Analysis of the data indicates a transfer of free water from maternal to fetal circulation at the placenta Events on the fetal side of the placental circulation concur with events on the maternal side

3 Water balance between maternal and fetal organisms is discussed in terms of placental transport of free water fetal secretions amniotic fluid and reabsorption of free water at the amniochorionic membrane

The authors would like to express their appreciation to Dr E L Corey for his aid and encouragement during the pursuit of this project

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TRANSBUCCAL ADMINISTRATION OF PITOCIN *

Thomas F Dillon

In 1926 Hofbauer and associates investigated the oral administration of pituitary extracts [1] Two main considerations influenced their search for new channels through which pituitary extract could be absorbed first the opinion that the best results followed the repeated administration of small dosages and second the possibility of withdrawing the hormone when the desired response was present With the use of a solution of pituitary extract dissolved in sugar water they induced and/or stimulated labor in 31 patients The patient held this solution under the tongue for several minutes and then swallowed it It had been established that the hormone was inactivated in the gastrointestinal tract and thus they depended upon the sublingual absorption for effect [2, 3, 4] Because their results were not ideal they then turned to the application of the pituitary hormone to the inferior turbinate and concluded that this technique was more efficient [1, 5, 6, 7] Over the years to the present time the parenteral and principally the intravenous route has become the accepted and safest method of administration of pituitary hormones in obstetrical practice [8, 10, 11, 12] These developments and advances were in no small way influenced by the purification of the oxytocic hormone of the posterior pituitary [13, 14, 15]

Stimulated by four factors in addition to those that prompted Hofbauer we began a program designed to reinvestigate the oral administration of Pitocin These factors are the availability of newer and purer pituitary preparations the development of a Pitocin linguet the very real need for an agent to aid milk let down in the lactating patient and the still existing requirement for a simpler yet safe method of administering this oxytocic agent It has been well established that Pitocin and pure oxytocin either

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natural or synthetic, will effect induction and/or stimulation of labor and stimulate milk let down in the postpartum patient[9] Our interest was thus *to attempt to reestablish the transbuccal or sublingual route as a simple, safe* and efficient method for administration of these hormone fractions

METHOD

Preliminary trials were conducted on 8 normally lactating patients to whom one or more Pitocin linguets of 10 units strength were administered transbuccally The transbuccal route was chosen on the basis of previous trials that indicated the hormone could be administered as efficiently through this membrane as sublingually, and secondly, because of less salivary response and perhaps less loss to the gastrointestinal tract The results indicated that the average dosage to stimulate milk secretion was of the order of 20 units This compared to averages of 0 5 to 2 units parenterally effecting the same response The technique of administration is identical with other transbuccal therapy in that the tablet is placed and absorption occurs in the buccal space The observed activity is the same as parenterally administered oxytocin except for the time of absorption of 30 minutes and the critical dosage that is at least 10 times as large The slow absorption of the hormone acts as a safety factor in that the response can be titered as the medication passes through the buccal barrier The patient simply spits out the tablet when the galactorrheic or uterine response is optimum

To date we have administered the Pitocin linguet 84 times to 35 patients These 84 administrations have resulted in evidence of milk secretion 33 times breast symptoms or uterine cramps 48 times and no response in 24 instances In *these cases, the end point in judging success* was the appearance of milk, while subjective symptoms of breast tightening with easier subsequent nursing or uterine cramps were taken into account

DISCUSSION

Analyzed differently the data on the following table indicate the response per postpartum day

It is to be noted that most of the instances of no response occurred in the immediate postpartum period before milk was present in the breast In this series, 10 instances of early breast engorgement were encountered Breast softening and alleviation of symptoms occurred in these patients in dosages of similar magnitude We have had no experience with late engorgement or mastitis in this series The dosage in units listed in the table as odd numbers and fractions are accounted for by the loss of potency of the linguets as a function of time This problem necessitated frequent reassay of the tablets and constitutes a condition that has been partially overcome by the development of a more stable preparation

As a corollary to the above study we have initiated a program for the administration of transbuccal Pitocin for the induction and stimulation of labor in selected patients This has been undertaken for the reasons cited above which may bear repitition namely, the existing requirement for a simpler yet safe route of administration, and the potential use of this method in those patients in whom premature induction is indicated wherein the process of preliminary cervical effacement could be easily, rapidly and safely carried out and the induction then initiated by this or the intravenous method To

Table 1

	MILK	SYMPTOMS	NONE
POSTPARTUM DAY 1			
6 administrations of 17.4 units	0	4	2
6 administrations of 26.1 units	0	1	5
POSTPARTUM DAY 2			
3 administrations of 17.4 units	1	2	0
3 administrations of 26.1 units	0	1	2
3 administrations of 34.8 units	0	1	2
1 administration of 43.5 units	0	1	0
POSTPARTUM DAY 3			
8 administrations of 17.4 units	5	7	1
3 administrations of 26.1 units	2	1	0
4 administrations of 34.8 units	0	2	2
1 administration of 43.5 units	1	0	0
3 administrations of 52.2 units	2	1	0
POSTPARTUM DAY 4			
4 administrations of 17.4 units	3	3	0
2 administrations of 26.1 units	2	0	0
3 administrations of 34.8 units	1	2	1
3 administrations of 43.5 units	2	2	0
5 administrations of 52.2 units	2	1	2
POSTPARTUM DAY 5			
3 administrations of 17.4 units	1	2	0
3 administrations of 26.1 units	2	1	1
3 administrations of 34.8 units	2	3	0
4 administrations of 43.5 units	2	1	2
4 administrations of 52.2 units	3	3	0
POSTPARTUM DAY 6			
1 administration of 26.1 units	0	1	0
3 administrations of 34.8 units	1	2	1
4 administrations of 52.2 units	1	0	3

date, we have administered Pitocin linguets to only 4 patients No conclusions can be drawn from this small series However, utilizing tablets of 27.7 units strength and administering successive linguets as the previous one was absorbed we have been able to produce regular uterine contractions at constant intervals Briefly, the first patient was a normal multigravida at term who was considered ready for induction This patient received one half of one linguet or 13.9 units 27.7 units ½ hour later, 41.6 units one hour and 15 minutes later, and then 55.4 units on successive half hours to a total of 194 units The response was regular 5 minute contractions over a period of 5 hours at which time the medication was discontinued Equivocal effacement of the cervix had occurred The patient spontaneously developed labor two days later and delivered normally The second patient was a 15 year old primagravida with mild preeclampsia Induction was indicated because of the complication

The patient was at term. Administering Pitocin in the same manner beginning with one half of one tablet (13.9 units) and progressing through dosage ranges of 27.7, 55.4, and 83.1 units respectively every half hour or when the previous tablets were absorbed, contractions of an irregular 5 to 8 minute pattern were produced of poor 20 to 40 second quality over a 3 hour period. During the second 3 hours the patient was placed on intravenous Pitocin to which she responded with 3 minute contractions of 40 second duration to a maximum concentration of 4 units. After 3 hours the induction was abandoned and re-instituted the following day. The patient delivered in 4 hours with 12 units of Pitocin in 500 cc. of 5% dextrose and water administered intravenously. The third and fourth patients were normal multigravidae similar to the initial patient studied with the exception that the membranes had ruptured prematurely in one. Each patient responded with contractions of satisfactory magnitude. However, the patient with intact membranes ceased uterine activity when the Pitocin was stopped and it was necessary to administer intravenous Pitocin to the patient with ruptured membranes.

No conclusion can be drawn as yet. It is obvious that the hormone will be absorbed through the buccal membrane and produce a uterine response. However, it is also obvious that the technique must be improved to effect a greater absorption of Pitocin to provide adequate uterine stimulation.

SUMMARY

1. A preliminary report is presented on the initial data utilizing a Pitocin linguet for the stimulation of lactation and for the induction and stimulation of labor.

2. The oral administration of Pitocin by means of a transbuccal linguet will effect milk let-down in the lactating patient. It will also relieve symptoms of early engorgement.

3. Transbuccal administration of Pitocin effects uterine contractions at term. These contractions are clinically indistinguishable from but not as efficient as those produced by intravenously administered Pitocin.

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ADRENAL CORTICAL HORMONE OUTPUT IN CEREBRAL INJURY AND NEOPLASM *

ROBERT G. FISHER, J. H. COPENHAVER, JR. AND IRMELI NAUKKARINEN

The activity of the adrenal cortex has been studied extensively in general surgical problems but little attention has been paid to alterations of adrenal cortex output in neurosurgical conditions. This preliminary report is concerned with the output of adrenal cortical hormones as measured by urinary excretion in cases of cerebral injury, cerebral neoplasm, and cancer of the breast undergoing hypophysectomy. Continuing studies are in progress to correlate serum, urinary and cerebrospinal fluid electrolyte levels with urinary output of 17-hydroxycorticosteroids, 17-ketosteroids, and aldosterone.

METHOD

Twenty-four hour urine samples were collected and either frozen or extracted immediately. The 17-hydroxycorticosteroids were measured by a modification[1] of the method of Porter and Silber.[2] The 17-ketosteroids were determined by the method of Pincus and Pearlman.[3] Aldosterone was extracted and measured by the modified method of Nowaczynski, Koiw, and Genest.[4, 5, 6]

Case 1. A 19 year old white male sustaining a closed head injury was admitted in deep coma and with Cheyne Stokes breathing. Trephination was done under light nitrous oxide anesthesia shortly after admission. No abnormality of the brain was detected. The patient remained unconscious for 5 days and psychotic for the next 5 days. Six weeks after admission he was judged normal by both psychiatric and neurological standards.

Case 2. An 8 year old white male was admitted 24 hours after sustaining a closed head injury and a fracture of the femur. Trephination under light pentothal nitrous oxide anesthesia disclosed bilateral subdural hematomas which were removed. The brain was swollen. The patient remained decerebrate for 7 days and regained consciousness 2 weeks after admission. He was of normal intelligence 3 months after the accident with no neurological defect.

Case 3. A 32 year old white male, blind in the left eye, was admitted and diagnosed as having a chromophobic adenoma of the pituitary. This was removed under pentothal nitrous oxide anesthesia. The patient was given cortisone pre and post operatively and had no postoperative complications. He has regained complete vision of the eye and has no endocrine deficiency.

Case 4. A 14 year old white female was admitted with aphasia, alexia, and a right hemiparesis due to a malignant cystic glioma in the left frontoparietal region which was subtotally removed. She had moderate swelling of her scalp flap after the operation. She subsequently recovered her motor function, speech, and reading ability, but died 6 months later of a recurrence. No autopsy was done.

Case 5. A 70 year old woman underwent hypophysectomy for carcinoma of the breast which had been removed 2½ years previously. Prior to hypophysectomy she was found to have metastases involving the anterior chest, left axilla, anterior

* From the Dartmouth Medical School, the Hitchcock Clinic, and the Department of Zoology, Dartmouth College, Hanover, New Hampshire. Supported by Grant B 916 (R) from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

mediastinum and left humerus These areas were all worse symptomatically under a trial of stilbestrol and hypophysectomy seemed justified For 6 months post operatively the patient has gained weight and her metastatic areas are smaller and asymptomatic

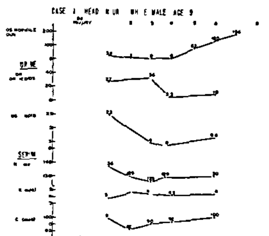


Fig 1

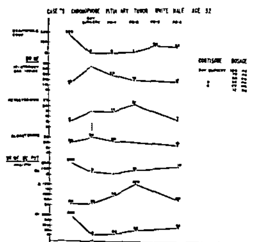


Fig 2

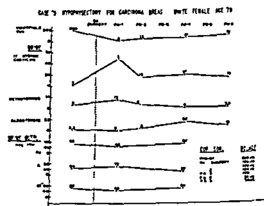


Fig 3

RESULTS

All of our cases were done under light nitrous oxide pentothal anesthesia Thus, these results may represent the stress of neurosurgery upon the patient However, these values are being presented because they represent the reactions of neurosurgical patients Studies are now in progress on the brain injured patient for whom surgical therapy is not necessary

The two cases of cerebral trauma (cases 1 and 2) disclosed elevated steroid levels after the combined trauma of the injury and anesthesia All steroid levels were found elevated in the second case although the complicating factor of a fractured femur cannot be neglected However, the aldosterone level of 10 7 gamma in an 8 year old boy seems quite high

The third case of a destructive pituitary tumor disclosed no preoperative steroid deficiency and none was shown postoperatively although this may have been masked by supportive therapy with cortisone His serum electrolyte pattern showed fairly close correlation with his aldosterone excretion in that the high level of aldosterone excretion was accompanied by sodium retention

Case 4 was that of a malignant brain tumor in a young girl Her preoperative

studies disclosed no pituitary adrenal deficiency Her postoperative serum electrolytes were confusing the potassium paralleling the sodium level rather than being inversely related although aldosterone levels rose only to 7 8 gamma on the first postoperative day

Case 5 was a woman of 70 who has had an excellent record subsequent to hypophysectomy for carcinoma of the breast We found no immediate rise in aldosterone postoperatively but oddly enough found a rather marked elevation of this level on her fifth and sixth postoperative days Undoubtedly her cortisone dosage influenced her urinary 17 hydroxycorticoid levels

DISCUSSION

This study has been undertaken to determine what role the adrenal cortex may play in alteration of fluid and electrolyte levels of the cerebrospinal fluid urine and serum in various neurosurgical lesions Cerebral swelling and altered serum electrolyte levels are well known to occur with intracranial trauma whether this be the result of blunt injury or surgical therapy

The report of Smolik Nimecurt and Nash[7] indicated that the eosinophile circulating response was a good prognostic factor in head injuries If the count did not rise above 0 within 96 hours the patient was likely to die regardless of the pathology involved Grenell and Mendelson[8] investigated the effect of adrenal steroids on the cerebrovascular permeability and found the blood brain barrier resistance was lowered with extravasation into the brain of intravenously administered trypan blue Hume[9] has found that ACTH secretion with resultant eosinopenia after trauma was the result of median eminence stimulation and if this was destroyed no ACTH secretion occurred

For many years unusual serum electrolyte patterns after primary intracranial disease have been reported although the mechanism is unknown Allott[10] reported 5 cases of hypernatremia and hyperchloremia with brain lesions and no kidney disease Sweet *et al*[11] reported a patient with hypernatremia hyperchloremia hyperglycemia and azotemia after brain injury No steroid studies were made Because of these reports our interest in aldosterone has been stimulated Very little work has been reported to date concerning the effect of cerebral injury on neurosurgical procedures upon aldosterone excretion and related electrolyte balance Llaurado[12] has reported an immediate rise in aldosterone excretion following surgery No neurosurgical cases were included in his or other studies In view of the work of Rauschkolb and Farrell[13] implicating the diencephalon in the control of aldosterone excretion the study of the results of neurosurgical trauma as it affects aldosterone excretion and electrolyte balance will be very informative

CONCLUSION

A study is under way to investigate the role of the adrenal cortical hormones in cases of intracranial injury and neurosurgical intervention All cases presented were influenced by the stress of the surgical procedure but the adrenal cortex responds in typical fashion with elevation of all hormone levels after surgery with some variations found in the levels of aldosterone

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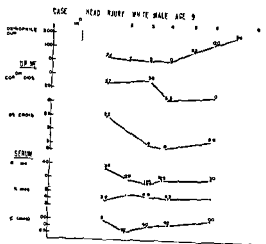


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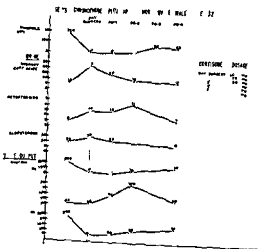


Fig 2

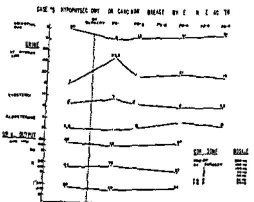


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Case 5 was a woman of 70 who has had an excellent record subsequent to hypophysectomy for carcinoma of the breast. We found no immediate rise in aldosterone postoperatively but oddly enough found a rather marked elevation of this level on her fifth and sixth postoperative days. Undoubtedly her cortisone dosage influenced her urinary 17-hydroxycorticoid levels.

DISCUSSION

This study has been undertaken to determine what role the adrenal cortex may play in alteration of fluid and electrolyte levels of the cerebrospinal fluid, urine, and serum in various neurosurgical lesions. Cerebral swelling and altered serum electrolyte levels are well known to occur with intracranial trauma whether this be the result of blunt injury or surgical therapy.

The report of Smolik, Ninecurt, and Nash [7] indicated that the eosinophile circulating response was a good prognostic factor in head injuries. If the count did not rise above 0 within 96 hours, the patient was likely to die regardless of the pathology involved. Grenell and Mendelson [8] investigated the effect of adrenal steroids on the cerebrovascular permeability and found the blood-brain barrier resistance was lowered with extravasation into the brain of intravenously administered trypan blue. Hume [9] has found that ACTH secretion with resultant eosinopenia after trauma was the result of medican eminence stimulation and, if this was destroyed, no ACTH secretion occurred.

For many years unusual serum electrolyte patterns after primary intracranial disease have been reported although the mechanism is unknown. Allott [10] reported 5 cases of hypernatremia and hyperchloremia with brain lesions and no kidney disease. Sweet *et al.*[11] reported a patient with hypernatremia, hyperchloremia, hyperglycemia, and azotemia after brain injury. No steroid studies were made. Because of these reports, our interest in aldosterone has been stimulated. Very little work has been reported to date concerning the effect of cerebral injury on neurosurgical procedures upon aldosterone excretion and related electrolyte balance. Llaurado [12] has reported an immediate rise in aldosterone excretion following surgery. No neurosurgical cases were included in his or other studies. In view of the work of Rauschkolb and Farrell [13] implicating the diencephalon in the control of aldosterone excretion, the study of the results of neurosurgical trauma as it affects aldosterone excretion and electrolyte balance will be very informative.

CONCLUSION

A study is under way to investigate the role of the adrenal cortical hormones in cases of intracranial injury and neurosurgical intervention. All cases presented were influenced by the stress of the surgical procedure, but the adrenal cortex responds in typical fashion with elevation of all hormone levels after surgery with some variations found in the levels of aldosterone.

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OBSERVATIONS ON THE USE OF UREA IN RHESUS MONKEYS *

MANUCHER JAVID AND JON ANDERSON

The effectiveness of urea in lowering intracranial and intraocular pressure has been demonstrated in 400 patients The majority received this agent intravenously in 30% concentration The purpose of this investigation was to ascertain the following points 1) The optimum rate of administration of lower concentrations of urea 2) The most desirable vehicle for the administration of urea 3) Effect of subcutaneous injections of urea

METHOD

Adult female Rhesus monkeys (average weight 4 5 kg) were used The animals were anesthetized with intramuscular nembutal 42 mg /kg initial dose Supplementary doses of 35 mg were given when necessary every 2 to 3 hours Urea concentrations ranging from 2 to 30% were employed in these studies with a dosage of 1 to 1½ gm /kg body weight In the first group cerebrospinal fluid pressure measurements were carried out A specially built apparatus was used to control the rate of intravenous injections The animals were placed in the prone position on a specially built table with head holder After the usual preparation a 20 gauge needle was inserted in the cisterna magna and connected to a bubble manometer

In the second group blood and urinary studies were carried out before and at frequent intervals after the urea administration

In the third group intraocular pressure measurements were obtained every 30 minutes with the monkey in the supine position using a McLean tonometer Cerebrospinal fluid pressure measurements were done on one monkey using the same method as above

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* From the Department of Neurosurgery University of Wisconsin Medical School Supported in part by the Wisconsin Alumni Research Foundation

RESULTS AND COMMENTS

Rate Studies. Earlier studies in human subjects [1, 2, 3] showed that there was a consistent and marked drop in cerebrospinal fluid pressure to levels which were substantially below normal. This occurred within an hour or two following urea administration. Clinically, it was felt that there was no point in reducing the intracranial pressure to such an extent (often to levels below atmospheric) except during craniotomy. With this thought in mind, it seemed desirable to reduce cerebrospinal fluid pressure to near normal levels and maintain it there for a longer period of time. In order to achieve this effect two methods were employed: 1) smaller doses of 30% urea at frequent intervals (every 3 to 4 hours), and 2) lower concentrations of urea 3.3 to 20%). Although repeated injections of 30% urea could bring about satisfactory levels, it did not seem desirable because of multiple venous punctures. Furthermore, it was hoped that one might be able to administer 5% urea solution as a substitute for fluid intake during the period when control of increased intracranial pressure is necessary. In case 41,[3] when a concentration of 3.3% urea was administered over an 8 hour period, it caused a rise in cerebrospinal fluid pressure. Further observation, however, indicated that when urea was administered in 5, 7½, and 10% concentrations to patients, the degree of cerebrospinal fluid pressure drop corresponded with the rapidity of administration of fluid. That is to say, when urea was injected slowly the cerebrospinal fluid pressure rose, whereas, when it was administered rapidly the pressure fell (Figure 1).

In order to investigate this point systematically 2.5%, 5%, 10% and 30% concentrations of a standard amount of urea (one gm./kg. body weight) were injected into the monkeys. The rate of injection in each concentration varied from 15 minutes to 120 minutes. In Figure 2 it is clearly demonstrated that the degree of cerebrospinal fluid pressure changes are proportional to the amount of urea administered during a given period of time, irrespective of the concentration used.

Vehicle Study. In all the intravenous administrations originally done, in collaboration with the late Dr. Settlage, a combination of 30% urea and 5% dextrose in water was used.[1, 2, 3, 4] This was well tolerated with the exception of occasional hemoglobinuria, observed when urea was administered to patients under general anesthesia during craniotomy. The same phenomenon has been observed in monkeys but not in dogs. The authors, in search for an

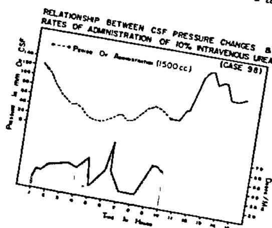


Fig. 1

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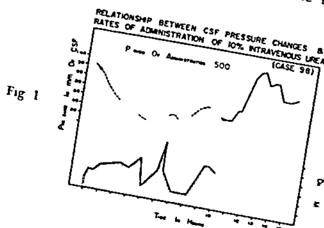
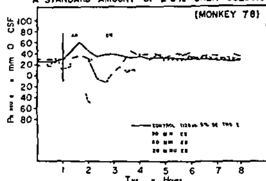
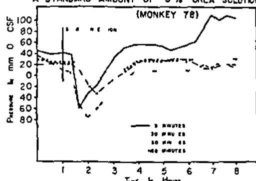
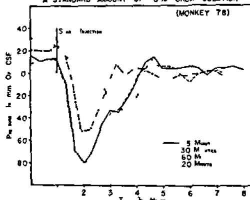


Fig 1







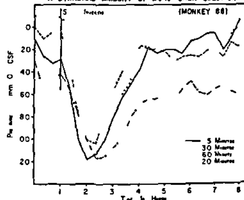


Fig 2 The degree of cerebrospinal fluid pressure changes are proportional to the amount of urea administered during a given period of time A) Note The control consisted of 5% dextrose in water administered in 30 minutes since it was felt that the volume of 2.5% urea solution administered over a 15 minute period would be excessive D) In 30% concentration cerebrospinal fluid pressure was maintained for a longer period of time when administered in the course of 60 minutes

ideal solvent, employed a series of 11 vehicles for the 30% urea solutions which were tested in Rhesus monkeys

Each monkey received a dose of 1 gm /kg body weight in the course of 30 minutes Urinary studies consisted of accurate measurement of output, inspection of color hemoglobin determinations and cell count Plasma hemoglobin was also determined Samples were taken every 30 minutes Average length of the studies following the urea administration was 5 hours Of all the vehicles employed 10% invert sugar proved the most satisfactory This was the only combination which did not produce hemoglobinuria There was some degree of hemoglobinuria from slight to very marked in the other 10 solutions ranging in this order 10% dextrose in water, 5% dextrose in saline, 4/5 saline normal saline, 5% fructose, 10% fructose 5% dextrose in water, 1 866 gm % sodium lactate 5% invert sugar, distilled water

It should be pointed out that the findings in many of the above solutions were very similar and this order should be considered at best, of relative significance with the exception of the two extremes, 10% invert sugar and the distilled water Of interest is the fact that the urine cleared in all cases before the discontinuation of each study Studies carried out on human subjects indicated that, for all practical purposes, the 10% invert sugar and 5% dextrose

in water are equally desirable 10% invert sugar having a slight advantage for the lack of occasional hemoglobinuria which was observed with 5% dextrose in water

Subcutaneous Injections Intravenous and oral administration of urea have both proven to be effective [1,2,4,6] Subcutaneous injection of 2% to 10% urea, dissolved in 5% dextrose in water was well tolerated in a few patients on whom this route of administration was used A total of 21 intraocular pressure measurement studies were carried out in 3 Rhesus monkeys using 10 and 30% urea (dose of 1 5 gm /kg body weight) In one of these monkeys the effect of similar amounts of subcutaneous urea were compared with intravenous All monkeys tolerated the subcutaneous injections well and there was no necrosis or sloughing of skin at the site of injection Urea was found to be effective in lowering both the intraocular and the cerebrospinal fluid pressures although not to the extent achieved by the intravenous route The efficacy of subcutaneous administration was increased by the use of Alidase and a slower rate of injection Figure 3 shows the results of one of the studies Figure 4 illustrates the comparison of intravenous and subcutaneous urea in

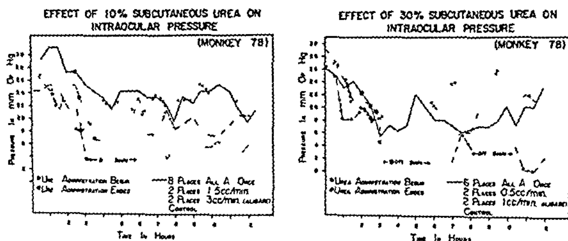
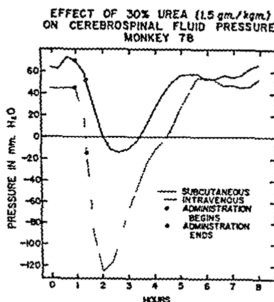


Fig 3 Reduction of intraocular pressure was increased with slow injection and the use of Alidase Of interest was the marked reduction of intraocular tension in control study where urea was not used Other controls showed no significant change

Fig 4



the same monkey Clinically, our experience with subcutaneous injection of urea is limited It should be pointed out, however, that although higher con centrations of urea are tolerated in monkeys they may prove harmful in man We have not used concentrations higher than 10% in man and are hesitant to do so In any case only very occasionally will one have to resort to the sub cutaneous route Our studies, to date, would indicate that the subcutaneous route may be useful, but further investigation is warranted

CONCLUSIONS

1 The degree of cerebrospinal pressure drop is proportional to the amount of urea administered during a given period of time, irrespective of the con centration used

2 Invert sugar 10% is the vehicle of choice to make a 30% solution of urea for intravenous administration to monkeys

3 Subcutaneous administration of urea in monkeys is well tolerated It is effective in reducing intraocular pressure It will produce reduction of cerebro spinal fluid pressure, although not as markedly as the intravenous route

The authors wish to thank Professor Frank Kozelka who made all the laboratory determinations and the Baxter Laboratories Morton Grove Ill who supplied us with the urea and the vehicles employed

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REPEATED LOADING TESTS OF THE LUMBAR SPINE *

A Preliminary Report

W G HARDY, H R LISSNER, J E WEBSTER AND E S GURDJIAN

The purpose of this investigation is to determine the effect of repeated axial compression and repeated transverse bending upon the intervertebral discs and bones of the lumbar spine Earlier investigation by Hirsch [1] on the mecha nism of low back pain indicated that compression of a disc causes the nucleus pulposus to exert radial pressure on the annulus fibrosis, which is conse

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quently subjected to circumferential tensile strain Intervertebral discs are almost entirely compression resisting structures and it has been shown that the discs can statically adjust themselves to various mechanical demands placed upon them Bones however are frequently subjected to bending action as a result of which they are required to resist tensile and shearing stresses and strains in addition to compression Recent studies by Brown and Hansen[2] indicate that failure under axial compression takes place in the vertebral cartilaginous end plates and varies according to the condition of the bone rather than according to the condition of the disc

METHOD

The materials used were fresh and embalmed human cadaver lumbar spines In most instances a specimen consisted of 5 lumbar vertebrae and their intervertebral discs with annulus posterior longitudinal ligament laminae and flaval ligaments intact The posterior elements were removed from all but 2 of the spines utilized in the transverse bending tests One to 2 cc of radiopaque solution was injected into the intervertebral discs through the anterior aspect of the annulus The spines were x rayed before and after testing Embalmed spines were preserved in formalin and immersed in saline solution while being tested The fresh spines were kept refrigerated when not being tested Two were frozen and thawed but this had no apparent effect on the test results During testing the fresh specimens were kept immersed in normal saline and furacin solution Osteolysis occurred to some degree in every specimen during the period of preservation and testing This period varied from a few days to 5 weeks The radiodensity decreased even in the embalmed spines but not to the extent seen in fresh specimens One specimen was preserved and tested for 21 days in saline and penicillin solution and showed the least osteolysis

The spines were placed in a modified commercial fatigue testing machine capable of applying direct compression and transverse bending The load was applied through a compression spring calibrated in a universal testing machine and applied at 120 cycles per minute The free ends of the spine were surrounded by a cylinder filled with noncompressible dental plastic and were immobile The supports were 6 inches apart and transverse loads were applied at the ⅓ points producing constant bending (flexion) over the center ⅓ of the spine The spines were fixed in formalin after testing then sliced sagittally in ¼ inch sections and studied grossly

RESULTS

Axial Compression Tests Five specimens were subjected to axial compression tests and the major changes produced are tabulated in Table 1 The machine was adjusted for initial constant compressive loads varying from 50 to 100 lbs for different specimens and the cyclic load varied from 400 to 900 lbs The loads dropped off during the tests due to shortening of the spine and narrowing of the discs Periodic readjustment of the testing machine was made to bring the load back to its original value Loading was continued until fatigue failure occurred X rays after spine failure indicated compression fractures in one or more vertebra of each specimen If fracture of the body was severe the posterior elements also fractured X rays of Spine No 2 after the load had fallen off but before fatigue failure occurred indicated narrowing

Table 1 Axial Compression Tests

SPINE	AGE	SEX	INITIAL X RAY FINDINGS	LOADS APPLIED & TOTAL CYCLES		FINDINGS ON X RAY & CUT SECTIONS
No 1 Fresh	48	WM	No abnormal structural change Posterior elements present	80 to 785 lbs at	1 290 000	Nucleus pulposus ruptured 3–6 mm into dorsal plates of L2 3 & 4 Compression fracture of dorsal plate and body of L2 3 4 & 5 Severe collapse body of L4 & 5
No 2 Embalmed	64	WM	No abnormal structural change Posterior elements present	50 to 500 lbs Fell off to 450 at 50 to 500 lbs Fell off to 400 at 50 to 400 lbs Fell off to 350 at (Plastic broken) 50 to 500 lbs Fell off to 350 at 50 to 500 lbs (Loading spring broken) at	 30 000 59 500 134,500 393 400 539 000	Narrowed disc space at L2–3 & later L3–4 Discs moved anteriorly and later ruptured 2–3 mm into dorsal plates of L3 & L4 Annulus intact Compression fracture dorsal plate of L3 & L4 finally
No 3 Embalmed	60	CF	Slight dorsal protrusion of L4–5 disc Posterior elements present	50 to 750 lbs Fell off to 400 at 50 to 750 lbs Fell off to 650 at 50 to 750 lbs to Load held	 141 100 372 300 401 200	All discs pushed anteriorly and annulus protruded 1–2 mm Annulus not ruptured Compression fracture body of L3 vertebra L4–5 annulus then protruded 3–4 mm anteriorly but not rupt
No 4 Embalmed	72	WF	Old compression fracture L4 body Slight dorsal protrusion of L2 & L3 discs Posterior elements present Osteoporosis	50 to 900 lbs Shortened rapidly	200	Nucleus pulposus moved posteriorly but did not rupture Annulus intact Severe compression fractures of L3 & L4 bodies & posterior elements fractured off L4
No 5 Embalmed	56	WM	No abnormal structural change Posterior elements present	100 to 900 lbs Fell off to 500 at 100 to 900 lbs Fell off to 500 at	 181 400 182 300	Discs not ruptured Annulus intact Compression fracture of body of L4 anteriorly

Table 2 Transverse Bending Tests

SPINE	AGE	SEX	INITIAL X RAY FINDINGS	LOADS APPLIED & TOTAL CYCLES		FINDINGS ON X RAY & CUT SECTIONS
No 6 Embalmed	60	WM	Posterior elements present	50 to 350 lbs. Fell off to 250 at	21 000	Transverse fracture of mid body of L4 vertebra anteriorly No ruptured disc Annulus not torn
No 8 Fresh	56	CF	Dorsal protrusion of annulus at L3–4 & L4–5 Posterior elements present	50 to 350 lbs. Fell off to 200 at	63,800	Fracture of pedicle and inferior articulating facet of L3 This next became a complete fracture and the body of L3 separated from the disc, while L2 disc bulged dorsally Finally the annulus of L3–4 tore dorsally & the disc ruptured posteriorly
				50 to 350 lbs. Fell off to 200 at	128 400	
				[illegible]0 to 3[illegible]0 lbs Fell off to 0 at	153 400	Same at L2–3 Torn through the cartilagenous plate
No 9 Fresh	57	WF	Posterior elements removed Small dorsal protrusion of L4–5 disc	50 to 300 lbs Fell off to 100 at	40,500	Cartilagenous plate of L3 torn off body of L3 posterior and inferiorly Disc and annulus torn on tension side L4–5 unchanged
				0 to 200 lbs Fell off to 0 at	41,400	
No 10 Fresh	73	WM	Posterior elements removed Spine is grossly weak and unstable Very osteoporotic	0 to 300 lbs Fell off to 0 at	500	Transverse fracture through body of L2 anteriorly Annulus L2–3 torn posteriorly and disc shifted dorsally but not ruptured
No 11 Fresh	64	CF	Small dorsal protrusion L4–5 disc. Posterior elements removed Patient died of peritonitis	0 to 250 lbs Fell off to 80 at	81 400	Bodies of L2 & L3 pushed apart by gas formation in nucleus pulposus Disc degenerated Protrusion of L4–5 disc 3 mm dorsally Annulus intact L2–3 annulus torn dorsally and disc pushed posteriorly but not ruptured General diffusion of radiopaque solution into vertebral bodies

Table 2 Continued Transverse Bending Tests

SPINE	AGE	SEX	INITIAL X RAY FINDINGS	LOADS APPLIED & TOTAL CYCLES		FINDINGS ON X RAY & CUT SECTIONS
No 12 Fresh	59	WF	Posterior elements removed No abnormal structural change	0 to 200 lbs to Not run to complete failure	17 300	Dorsal tear in annulus at L2-3 and L3-4 No rupture of disc No fractures
No 13 Fresh	61	WM	Posterior elements removed Interligamentous bone L4-5 laterally	0 to 150 lbs to Not run to complete failure	20 700	No ruptured discs Each annulus intact Transverse fracture of posterior mid body of L3 & L4 involving cartilagenous plates
No 14 Fresh	42	CF	Posterior elements removed No abnormal structural change	0 to 100 lbs to 0 to 100 lbs to 0 to 100 lbs Fell off to 50 at	17 100 32 000 38 500	No change in bones or discs at first except osteolysis Later each annulus became very loose and flexible especially L2-3 and L3-4 Annulus not torn No fractures occurred Discs did not rupture
No 15 Fresh	58	WM	Posterior elements removed No abnormal structural change	0 to 150 lbs Fell off to 125 at 0 to 170 lbs Fell off to 25 at	 10 300 54 600	No change upon X ray Annulus at L2-3 L3-4 & L1 2 became very flexible Sections showed transverse tears through cartilagenous plate and discs but no rupture

of the disc spaces but gave no good indication of what structural changes occurred at the time of failure. Spine No. 4 was very osteoporotic and fatigued rapidly. Specimen No. 1, the only fresh spine compressed, was much more resilient than the fixed spines and withstood the largest number of load repetitions before failing. Study of the sliced sections confirmed the x-ray findings. In addition, cracks in the vertebral cartilaginous plates and displacement of the plates into the vertebral bodies were found which were not apparent upon x-ray examination. This failure was associated with crumbling of the underlying bone trabeculae and shifting of the nucleus pulposus into these defects. Rupture of the nucleus pulposus into the dorsal plates was most marked at the centrum. The radiopaque solution would frequently leak into the vertebral body after compression of the spine to failure. It would also spurt out of tiny openings in various parts of the vertebral body. The annulus was not observed to be ruptured in any of the axially repetitively compressed spines.

Transverse Bending Tests. Nine specimens were subjected to transverse bending tests and the major findings are tabulated in Table 2. Spine No. 8, with its posterior elements present, sustained higher loads than those borne by other fresh spines with these elements removed. The articulations fractured and separated before the annulus bulged dorsally, and the vertebral body was torn horizontally through the cartilagenous plates. Spines No. 9, 10 and 11 received smaller loads and fewer cycles. Transverse fractures of the vertebral bodies resulted, associated with tears in the posterior aspect of the annulus and damage to the cartilagenous plates. The nucleus pulposus shifted dorsally toward the torn annulus but did not rupture unless gross avulsion occurred. Spines No. 12, 13, 14 and 15 received lower values of bending moment and were examined before total failure of the specimen occurred. Dorsal tears in the annulus occurred without rupture of the nucleus pulposus. The annulus became very flexible with loosened peripheral attachments and the specimens seemed unstable. However, fractures parallel to the cartilagenous plates occurred in the vertebral bodies with or without an intact annulus.

CONCLUSIONS

On the basis of the tests conducted so far there is no evidence that repeated loading will produce herniation of the intervertebral disc into the spinal canal unless fracture or avulsion occurs. Spines tested at the lower loading ranges in both direct axial compression and transverse bending showed no rupture of the nucleus pulposus through the annulus fibrosis. Repeated compression does not appear to produce rupture through the annulus. With very high loads rupture of the nucleus pulposus into the adjacent vertebral body can occur.

The spine (bones) itself proved to be very weak in the bending test but sustained greater loads with the posterior elements intact. The strength of the spine in bending appears to come from the composite action of the bones, muscles and ligaments. Failure of both bone and annulus occurs due to tension stresses or a tearing apart action. From the limited number of tests conducted to date no definite conclusions can be drawn but it appears that tears in the posterior annulus can be produced by repeated bending.

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CONFIRMATION TEST OF REACTION TO STRAIGHT LEG RAISING *

FRANK P SMITH

The common denominator in the evaluation of most cases of lumbar nerve root compression is the degree of limitation of straight leg raising The origin of this maneuver of flexing the hip joint when the lower extremity is fully extended has been accredited to Laseque As pointed out by Sjoquist [1] the technique of straight leg raising was not actually described by Laseque [2] in his pioneer article on sciatica in 1864 It was a pupil, Forst [3] who stated in his doctoral thesis that the principle of the straight leg raising test had been passed on to him by his teacher, Laseque All of this chronology has been reviewed by Woodhall and Hayes [4] as a preliminary to their emphasis on the value of Fajersztajn s well leg raising test [5] in the diagnosis of ruptured inter vertebral disc This test of reaction of the patient to straight leg raising contra lateral to the side of disorder is a valuable diagnostic test in many cases However, the complexity of the sciatic pain problem as indicated by Fincher [6] requires a broad range of resources in the armamentarium of the examiner to arrive at a clinical conclusion

Multiple variations of the straight leg raising test have been reviewed by DeJong [7] Negative response to these maneuvers eliminates any necessity for the confirmation test to be described in this paper Also a large portion of the cases of nerve root compression do not require confirmation of the patient s positive reaction to nerve root stretching tests However the incidence of subjective lumbosacral pain after an episode of skeletal trauma frequently raises the question of nerve root compression with the primary consideration being a possible herniation of intervertebral disc And thus there seems to be a growing group of cases who for reason of medico legal issue, hysterical re action or simple examination fright may present a problem of interpretation regarding any active manipulation The test to be described takes into con sideration that the apprehensive recumbent patient may claim aggravation of alleged sciatic pain with most any active stress test of spine or hip joint but does not intuitively resent return of the extremities to the position of re cumbency

METHOD

In the test for confirmation of reaction to straight leg raising the patient assumes the recumbent position and the steps are as follows (Fig 1) 1) The asymptomatic leg is placed in the position of full flexion at the hip and knee joints 2) The symptomatic leg held straight, is raised in hip flexion to the point of tolerance The angle of elevation will usually be higher than with the contralateral leg held straight 3) The patient is asked to return the asymp tomatic leg to full extension If this last step produces increase of the pain in the symptomatic leg the test is considered positive

DISCUSSION

Experience with this test has indicated that its primary value has been in recognizing the presence of nerve root compression in the case which has

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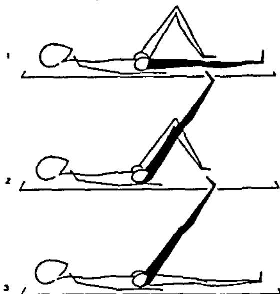


Fig 1 Shaded leg indicates side of primary sciatic pain

defied the ordinary phases of examination A negative confirmation test does not preclude the presence of nerve root compression A positive confirmation test may provide basis for further consideration of the possibility of an organic disorder when the superficial factors may indicate only an anxiety problem

SUMMARY

A test is described for confirming the reaction to straight leg raising in patients suspected of lumbar nerve root compression This procedure may be of help in cases which present problems of interpreting ordinary diagnostic measures

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CONFIRMATION TEST OF REACTION TO STRAIGHT LEG RAISING *

FRANK P SMITH

The common denominator in the evaluation of most cases of lumbar nerve root compression is the degree of limitation of straight leg raising The origin of this maneuver of flexing the hip joint when the lower extremity is fully extended has been accredited to Laseque As pointed out by Sjoquist [1] the technique of straight leg raising was not actually described by Lasèque [2] in his pioneer article on sciatica in 1864 It was a pupil Forst [3] who stated in *his doctoral thesis that the principle of the straight leg raising test had been* passed on to him by his teacher Laseque All of this chronology has been reviewed by Woodhall and Hayes [4] as a preliminary to their emphasis on the value of Fajersztajn s well leg raising test [5] in the diagnosis of ruptured inter vertebral disc This test of reaction of the patient to straight leg raising contra lateral to the side of disorder is a valuable diagnostic test in many cases How ever the complexity of the sciatic pain problem as indicated by Fincher [6] requires a broad range of resources in the armamentarium of the examiner to arrive at a clinical conclusion

Multiple variations of the straight leg raising test have been reviewed by DeJong [7] Negative response to these maneuvers eliminates any necessity for the confirmation test to be described in this paper Also a large portion of the cases of nerve root compression do not require confirmation of the patient s positive reaction to nerve root stretching tests However the incidence of *subjective lumbosacral* pain *after* an *episode of skeletal trauma frequently* raises the question of nerve root compression with the primary consideration being a possible herniation of intervertebral disc And thus there seems to be a growing group of cases who for reason of medico legal issue hysterical re action or simple examination fright may present a problem of interpretation regarding any active manipulation The test to be described takes into con sideration that the apprehensive recumbent patient may claim aggravation of alleged sciatic pain with most any active stress test of spine or hip joint but does not intuitively resent return of the extremities to the position of re cumbency

METHOD

In the test for confirmation of reaction to straight leg raising the patient assumes the recumbent position and the steps are as follows (Fig 1) 1) The asymptomatic leg is placed in the position of full flexion at the hip and knee *joints 2) The symptomatic leg held straight is raised in hip flexion to the* point of tolerance The angle of elevation will usually be higher than with the contralateral leg held straight 3) The patient is asked to return the asymp tomatic leg to full extension If this last step produces increase of the pain in the symptomatic leg the test is considered positive

DISCUSSION

Experience with this test has indicated that its primary value has been in recognizing the presence of nerve root compression in the case which has

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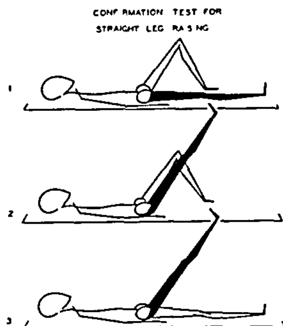


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GLOBUS PALLIDUS COAGULATION TECHNIQUE *

ORLANDO J ANDY

A simplified stereotaxic instrument † and a system of reference points have been devised for coagulation of the globus pallidus in the treatment of Parkinson's disease

METHOD

Stereotaxic Instrument. The instrument has three basic parts and when assembled, it is $3\frac{1}{2}$ inches in diameter and 2 inches in height (Fig 1) It contains a universal joint through which an electrode can be inserted This joint may be locked in place The base can be screwed into a burr hole made with a standard Hudson burr Before screwing the instrument into the burr hole threads are tapped in the skull A special wrench is then utilized to firmly screw the base of the instrument into the burr hole The same holes which were utilized for the placement of the wrench are then utilized for the placement of a sleeve which serves to hold the protractors The protractors which are at right angles to one another are readily attached to the sleeve and readily manipulated so that they may be placed parallel to the frontal and sagittal planes respectively The protractors contain notches placed at regular intervals for use in subsequent x ray measurements

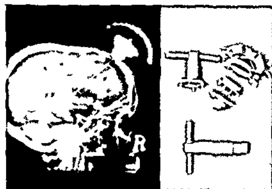


Fig 1 On the right are illustrated the various components of the simplified stereotaxic instrument which is screwed into the skull The lower instrument with the cross bar represents the tap and the upper one with the cross bar represents the wrench The three parts of the stereotaxic apparatus are so oriented that they will fit one into the other The left half of the figure illustrates the instrument and electrode in place

Operative Procedure. The operation is performed under local anesthesia The patient is placed on his back and the head positioned on an x ray cassette holder for subquent AP view of the skull The burr hole is placed in the frontal region approximately $7\frac{1}{2}$ cm posterior to the glabella and 2 cm lateral to the midline after infiltration with 0 5% xylocaine The anterior horn of the lateral ventricle is then tapped and the available spinal fluid is removed and replaced with air One should not inject the air under pressure If the fluid is removed and the air injected in equal amounts no ill effects will develop An attempt should be made to inject enough air to adequately visualize the region of the foramen of Monroe and thus the anterior commissure The stereotaxic instrument is then screwed into place so that the protractors which lie at right angles to one another, lie in the sagittal and transverse plane of the skull respectively AP and lateral x ray views of the

† Mr James Nelson of the Department of Pharmacology, constructed the original instrument It is now available through the Lawton Company Inc N Y

* From the University of Mississippi Medical Center, Division of Neurosurgery, Department of Surgery Jackson

skull are then taken in the operating room with a portable x ray unit Grid cassettes are used to obtain good definition The head must lie in a straight position during the x ray of the skull so that there will be no distortion of the ventricular system which would interfere with subquent measurements After the x rays are taken the desired point to be coagulated is determined with reference to a system of coordinates based upon a vertical line projected through the anterior commissure and the posterior third of the sella

Method of Measurement The atlas of Speigel and Wycis [1] is utilized for the coordinates A vertical line running between the anterior commissure and the posterior third of the sella is considered to be 25 mm in front of the posterior commissure A horizontal line is then made through the anterior commissure at right angles to the one just erected through the sella This line represents the horizontal zero The point of coagulation is calculated to lie between the planes 14 to 21 frontal 0 to —5 horizontal and 15 to 17 5 mm lateral

The use of the posterior third of the sella as one of the reference points was based on previous determinations made in brains having both the anterior and either the posterior commissure or the pineal gland identified According to these studies a line at right angles to the horizontal zero line which runs through the anterior commissure traverses the posterior third of the sella in over two thirds of the cases Maximum errors resulting from an incorrect sella point produces one to two millimeters variation in the horizontal plane Figure 2 illustrates variations which were found in a few x rays that were studied with reference to obtaining sella reference points by constructing a line at right angles to horizontal zero line which also runs through the anterior commissure The upper right corner figure represents a patient who had the greatest deviation One must add that in this patient the entire ventricular system was obviously posteriorly located when contrasted with the usual appearing ventriculogram Deviations of this magnitude can certainly be de tected during the procedure and allowances made X ray distortion must be taken into account when calculating for electrode placement

Stimulation Technique The electrode for stimulation and coagulation is bipolar It consists of a 20 gauge stainless steel tube with a noninsulated bare tip of 3 to 10 mm length An insulated copper wire runs through the tube and projects 1 mm from the tip The electrode is guided into the correct

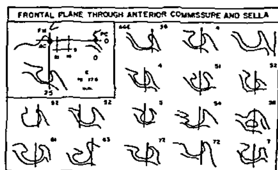


Fig 2 Anterior commissure sellar line zero line between the anterior and posterior commissure in a series of x rays in which both structures were identified was constructed A vertical line at right angles to that line and projected through the anterior commissure is illustrated with reference to the sella in 16 cases This line represents frontal plane plus 25 The insert in the left upper corner illustrates the essential points utilized in establishing this system of coordinates (FM—foramen of Monroe AC—Anterior commissure PC posterior commissure O—solid line horizontal zero 25 mm solid vertical line is the anterior commissure selar line O—verticle interrupted line is frontal zero plane 5 14 21 and 15–17.5 millimeters delineate desired area for coagulation)

position through a hole in the universal joint which is located in the center of the stereotaxic instrument Bipolar stimulation and recordings are done with a Grass square wave stimulator and electroencephalograph respectively Parameters of stimulation are 1 to 40 volts 20 to 70° cycles per second 1 millisecond pulse and 5 to 15 seconds duration Since the shaft of the electrode has a greater tissue contact than the central electrode the polarity is reversed to adequately evaluate the area being considered for coagulation Unipolar stimulation also is employed with the reference lead on the body Thus to fully evaluate the character of the response and to evaluate the influence of the stimulation upon the existing tremor or the rigidity, the parameters of stimulation the polarity and the position of the reference electrodes are varied Blood pressure changes are also recorded during stimulation Such evaluations are carried out in order to determine as closely as possible where the electrode is located and thus assist the operator in deciding whether this is the desired point for coagulation

Coagulation Technique Coagulation is done through the bare tip of the electrode barrel Standardization of the parameters employed for coagulation has been carried out on laboratory animals In general a Bovie setting of 30 for a period of 15 seconds produces a lesion which is 1½ to 2 mm in circumference beyond all points of the exposed surface of the electrode Thus an exposed tip which is 10 mm in length will make a lesion approximately 4 mm in diameter and 13 to 14 mm in length During the coagulation both heat and gases are allowed to escape through the barrel of the needle Before during and after the coagulation the patient is evaluated with respect to orientation speech motor and sensory functions

SUMMARY

A new and simplified stereotaxic instrument in addition to a sella point of reference have been presented in order to guide an electrode to a predetermined point in the globus pallidus The electrical stimulation and coagulation techniques employed are also discussed

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VENTRICULOBILIARY SHUNT: A NEW TREATMENT FOR HYDROCEPHALUS*

GEORGE W. SMITH, WILLIAM H. MORETZ AND WILLIAM L. PRITCHARD

Despite the fact that hydrocephalus is a condition about which we know the etiological factors and points of obstruction to the flow, the long account of multiple therapeutic procedures is evidence that a satisfactory method for drainage has yet to be devised. The logical approach of short circuiting the spinal fluid seems apparent and simple, yet the body at each turn has defied a completely satisfactory result. The many therapeutic trials have not been in vain: they, each in their shortcoming, have pointed toward the physiological principles necessary for a satisfactory procedure.

Current surgical approaches have been those of leading the continually produced cerebrospinal fluid away from the ventricular and subarachnoid pathways into a suitable absorbing area, thus reducing the intracranial hypertension. This report presents another method of diverting the spinal fluid into a suitable receptor organ, namely, the gallbladder.

It was necessary to anticipate certain problems in proposing such a procedure. These problems fall into two categories: mechanical and biological.

The mechanical problems concern: 1) the maintenance of one flow in the shunt with prevention of reflux of bile into the central nervous system; 2) the preservation of patency of the shunt mechanism.

The biological problems concern: 1) the reaction of the gallbladder and neighboring structures to the presence of a shunt appliance; 2) possible anatomical and physiological alterations secondary to this reaction.

In regard to the first problem, i.e., the prevention of reflux of bile into the CNS, it was proposed to construct a simple valve mechanism employing a bicuspid flapper type valve. After using various materials, designs and sizes, the present model evolved (Fig. 1).

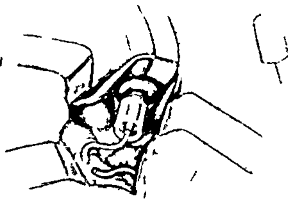


Fig. 1. Artist's drawing of the surgical exposure with the valve in place and anchored into the fundus of the gallbladder. The insert shows the valve.

There are three phases to this presentation: (a) the designing and making of a competent valve in the laboratory; (b) the animal experimentation to determine the feasibility of the gallbladder as a receptor organ for cerebrospinal fluid; (c) the clinical trial of the procedure.

* From the Medical College of Georgia, Augusta.

METHOD

Designing and Making of a Suitable Valve To determine the resistance of materials to the action of bile rubber lucite and polyethylene were placed into concentrated bile solution and allowed to be so exposed for several months The same experiment was carried out *in vivo* placing these materials into the bile of the functioning gallbladder of three healthy average sized mongrel dogs After 8 12 and 16 weeks respectively these animals were sacrificed and the materials inspected Rubber appeared to show deterioration in the continued presence of the bile The lucite and polyethylene showed no gross changes

The principle of the valve is that of a bicuspid flutter mechanism enclosed within a water tight cylinder for the purpose of exerting back pressure with closure Polyethylene tubing I D 070′ O D 110′ was compressed in a vise under heat to form two thin opposing leaflets at the terminus of the tube By wetting the polyethylene with propylene glycol one prevents the adhesion or fusion of the two valve leaflets Hydrostatic pressure of 50 to 80 mm is required for the valve to open At lesser pressures the valve will close thus affording a margin of safety for the prevention of regurgitation The valve is encased in a cylinder of lucite machined by lathe in which is incorporated the outflow end with two elevated ridges or collars for the securing of the purse string sutures All seams are snugly fitted to water tight tolerance The over all dimensions are length 2 7 cm diameter 9 cm and weight 1 5 gm (Fig 1)

To investigate the competency of such a valve experiments were performed in a series of 12 healthy average sized mongrel dogs The various experimental models of valves were aseptically installed in the gallbladders of these animals under general anesthesia The inflow end was left free in the peritoneal cavity

Clinical Trial Ten patients (Table 1) suffering from advanced obstructive hydrocephalus were treated by shunting the cerebrospinal fluid into the gall bladder with the valve in circuit As in the case of a clinical trial of any new procedure the patients selected were considered far advanced terminal hydrocephalics

In the communicating hydrocephalus the shunt was performed by a hemilaminectomy at the level of L2 with the tubing being led around the right flank laterally to the peritoneal cavity which was entered through a right subcostal incision The collar of the valve was placed into the tip of the fundus of the gallbladder and anchored by means of two subserosal purse string sutures Two of the internal hydrocephalus patients had a primary Torkildson procedure performed with a subsequent spinal subarachnoid biliary shunt The method of treating the patients with internal hydrocephalus was one of leading a polyethylene tube size 200 from the right lateral ventricle posteriorly over the right thorax in the subcutaneous layers or whenever feasible beneath a muscular layer to the right costal margin then advancing the tubing into the peritoneal cavity through a subcostal incision and fastening the collar of the valve into the fundus of the gallbladder (Fig 1) The majority of the patients were poor surgical risks For this reason in only 3 cases was a complete procedure performed primarily In the remainder of the patients a peritoneal shunt was performed as an initial procedure and after the patient

Table I. Patients Being Treated with this Method

INITIAL	SEX	AGE	ETIOL	TYPE	COURSE	RESULT	AUTOPSY	VALVE
M B	F	11 mo	Hydro anencephaly	int	Excellent w/ improved develop	Died 8 mo post shunt in status ep	Shunt working	unchanged
J A	F	23 yrs	Post Traum	com	Clinical course unchanged	Died in 3 mo pneumonia and decubiti	Shunt working	unchanged
T J	M	8 mo	Congen	int	Clinical course improved	Still living 2 years		
C W	F	7 mo	Congen	int	Necessary to unplug tube improved w/ relief	Still living 12 mo		
C B	M	6 mo	Congen	int	Died 8 hrs post op	Cardiac death	Shunt working	unchanged
B W	F	7 yrs	Post Traum	com	Relieved hydroceph improved develop	Still living 8 mo		
R H	M	8 mo	Post Infect	com	Temp improvement w/ subseq infect along tube	Still living 5 mo		
R P	F	29 yrs	Post fossa tumor	int	Relieved hydrocephalus	Died 3 mo from growth of tumor	Shunt working	unchanged
T N	F	2 mo	Arnold Chiari	int	Improved develop	Still living 2 mo		
L S	M	[illegible] mo	Toxoplasmosis	int	Improved develop	Still living 2 mo		

was in better condition, the anastomosis of the tube to the gallbladder with the valve in circuit was performed

RESULTS

Animal Studies. Seven dogs died or were lost due to technical operative difficulty or defective valves One dog died 8 days postoperatively of causes not related to the experiment but was suitable for study Four dogs lived 8 months or more in good health, showing no clinical signs of obstructive jaundice or other biliary disease, and were reexplored under general anesthesia prior to sacrifice

At reexploration, attention was directed to the following the effects of bile on the valve, the competency of the valve, the patency of the valve, and the anatomical changes in the gallbladder and neighboring structures

None of the valves became occluded, either by fibrotic process or by bile salt deposits There was no grossly discernible effect of bile on the physical characteristics of lucite or polyethylene The valve became firmly fixed in an envelope of fibrous tissue contiguous with the capsule of the liver This fibrotic response was localized, there being no generalized peritoneal reaction

The anatomy of the biliary tree was thoroughly explored and found unaltered by the presence of the appliance The appearance of the mucosa was normal with absence of chronic inflammatory cells

Bile was present in these gallbladders, and was usually concentrated, with no evidence of sludge or stone formation

Clinical Trials. (Table 1) Four patients died, all of whom had postmortem study In case 1, the valve was functioning The pathologist reported yellow staining of the ventricular wall with xanthochromic cerebrospinal fluid The other postmortem studies showed the valves functioning with no evidence of retrograde flow of bile No patient developed electrolytic imbalance no patient developed retrograde infection from the gallbladder nor did they show evidence of gallstones or biliary dysfunction This report is not intended to compare results in treating hydrocephalus but is rather intended to demonstrate the feasibility of shunting cerebrospinal fluid into the gallbladder and the effectiveness of this viscus as a receptor organ

DISCUSSION

The cause of failure of previously reported procedures has been due to the following 1) resultant meningitis 2) obstruction to the flow at the distal end of the prosthetic tube, 3) electrolyte imbalance due to continued loss of cerebrospinal fluid 4) hydrostatic imbalance of cerebrospinal fluid within the central nervous system, 5) interference with normal physiological process in the remaining organs of the body

To put the principles in an affirmative manner, the following must be presented *for a satisfactory shunt procedure to persist* 1) The receptor organ must be relatively sterile 2) Blockage of the shunt mechanism by fibrin or other material must be obviated 3) Opportunity must be provided for the resorption of water and electrolytes contained in the shunted cerebrospinal fluid 4) Physiological intracranial pressure should be maintained 5) The shunt mechanism must not interfere with other physiological processes or constitute a physical detriment to the patient

That the gallbladder might constitute a suitable receptor organ for a shunt

procedure seemed reasonable. The theoretical advantages of this organ are: 1) It is a relatively sterile organ, in the absence of biliary obstruction. 2)
ion of electrolytes and water in the
pressure within the biliary system
tend to maintain intracranial pressure at a suitable level. 4) The gallbladder is not an essential organ. 5) Bile is a lytic substance and prevents the formation of a fibrous reaction and tissues which will either plug the distal end of the tube or form a fibrous cyst.

SUMMARY

1. A bicuspid valve with one way flow made from lucite and polyethylene is competent to prevent the reflux of bile and can be tolerated by the gall bladder over a long period of time

2 Valves made of lucite and polyethylene are relatively durable and can withstand the application of bile over long periods

3 The gallbladder is biologically suited to continually receive, absorb and to conduct cerebrospinal fluid over long periods without evidence of electrolyte imbalance.

4. The lytic action of bile is effective in the prevention of obstructive fibrosis at the distal end of a prosthetic shunting tube.

THE TREATMENT OF COMMUNICATING HYDROCEPHALUS BY THE ABSORPTION OF CEREBROSPINAL FLUID BY THE MUCOSA OF AN ISOLATED SEGMENT OF ILEUM (MODIFIED ILEO ENTECTROPY) *

CHARLES G NEUMANN, THOMAS I. HOEN, AND DONALD A DAVIS

In the absence of a uniformly successful medical or surgical method for the treatment of congenital communicating hydrocephalus, an effort has been made to utilize the ileal mucosa to aid in the absorption of cerebrospinal fluid of an infant afflicted with such hydrocephalus Previous work has shown that the ileal mucosa is capable of facilitating the absorption of fluid in another pathological state in which excessive accumulation of water is a major problem, namely, the ascites of advanced hepatic cirrhosis [1] This suggested that a similar beneficial effect might be attainable for communicating hydrocephalus

In the patient under consideration, the diagnosis of hydrocephalus had been established at 2 weeks of age When the child had attained 7 months of age, the occipitofrontal circumference of the head measured 55 cm, in comparison with a normal size of approximately 43 cm [2] Pneumoencephalograms revealed that the hydrocephalus was of the communicating type. If an

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THE EFFECT OF HYPOTHERMIA ON ACUTE EXPERIMENTAL SUBARACHNOID HEMORRHAGE IN DOGS *

SEAN MULLAN AND CHARAS SUWANWELA

Subarachnoid hemorrhage from intracranial berry aneurysms is a disease with a formidable mortality Approximately 15% of patients die of their initial hemorrhage within 24 hours after admission to the hospital a further 15% die of that or of a subsequent hemorrhage within the first week and another 15% will similarly die within the next 2 or 3 weeks Within a month the mortality rate is thus 45% and in the months and years that lie ahead probably another 10% will die of a late recurrence Of the survivors many are partially or totally incapacitated by hemiparesis dysphasia diplopia mental deterioration and epileptic seizures

In the management of this distressing disease the tasks before us are to aid the patient to survive his initial attack and to prevent the subsequent hemorrhages to which he is so prone Much work has been done on the second of these problems within the last two decades by surgical obliteration of the aneurysm but toward the first task that of surviving the initial hemorrhage little has been accomplished We do not know the precise mechanism of death It could be the result of raised intracranial pressure or brain swelling or brain displacement by blood clot or perhaps it could be caused by vascular spasm or by a combination of these factors

If we could lower the intracranial pressure and reduce the brain swelling it would be reasonable to expect a lower mortality Rosomoff[1] has shown that the cerebrospinal fluid pressure of normal dogs falls to approximately one half its previous level as the animal is cooled from 37°C to 27°C He has also estimated that during this procedure the brain volume shrinks by 4% Botterell[2] and others have noted that human brains at operation under hypothermia are slacker than normal However Smith[3] has observed cerebral edema in a few of the cases which he and Fay have subjected to prolonged hypothermia (in an effort to control malignant disease) and Botterell[2,4] has had to remove a swollen frontal lobe both during and subsequent to hypothermic operations The control of raised intracranial pressure and brain edema by hypothermia is thus by no means established and towards an understanding of the problem we have subjected a number of normothermic and hypothermic dogs to a controlled subarachnoid hemorrhage and compared the survivals

METHOD

Mongrel dogs weighing about 7 to 8 kg each were used Under nembutal anesthesia through a small trephine hole in the vertex of the skull a fine polyethylene catheter with tantalum wire stylet was inserted through the brain substance until it encountered resistance in the region of the chiasmatic or interpeduncular cisterns It was then withdrawn 2 mm , anchored and several inches of the external end were coiled and buried beneath the skin The internal end lay beneath the pia in the region of the basal cistern and any fluid injected into the external end would burst through this pia and pour into the subarachnoid space Seven to 10 days later fibrous tissue had completely sealed

* From the Department of Neurological Surgery University of Chicago Aided by a grant from the Douglas Smith Foundation

the trephine hole leaving the intracranial cavity again water tight The animal was anesthetized (nembutal 35 mg /kg) and whole blood obtained from the femoral artery was injected in 6 to 9 equal quantities over a period of 20 to 45 minutes into the external end of the previously implanted catheter In the case of the hypothermic dogs the hypothermia was established before the hemorrhage was produced in the belief that if it were to be of value it should be available from the commencement of cerebral damage In most of the animals the blood spread throughout the subarachnoid pathways forming a thick clot in the basal cisterns In a few there was intracerebral hematoma and in a few the blood was mainly subdural The extent of the hemorrhage was measured in ccs of blood/kg of body weight

RESULTS

Animals in which less than 1 cc of blood/kg of body weight was injected usually survived (Group 1) Gradually increasing amounts were then used (Group 2) with occasional deaths until the 1 9 cc per kg figure was reached After this there was no survival

Next we attempted a similar experiment in animals cooled to 25°C or below Practically none survived (Group 3) The temperature in Group 4 was raised to the 27 to 30°C range and their results closely resembled Group 2 There were no survivals after 1 9 cc /kg This was disappointing If cerebral anoxia or acute brain swelling had been the cause of death we would have expected better results in the hypothermic animals

We next turned our attention to another possible cause of death—blood clot in the subarachnoid spaces Using heparinised blood in normothermic animals (Group 5) the lethal volume of blood was raised from 1 9 to 2 5 cc /kg This figure could not be exceeded when hypothermia was simultaneously employed (Group 6)

In ten dogs the blood entered the subdural space (Groups 7 and 8) instead of the subarachnoid space as intended There were only two survivors and both were hypothermic While this may suggest some beneficent role on the part of the hypothermia the numbers are too small to draw conclusions

Table 1 Subarachnoid Whole Blood

CC./KG	NORMOTHERMIC GROUP 2	HYPOTHERMIC GROUP 4
1 0	O	X
1 1	O	O
1.2	X	O
1 3		
1 4	X	O
1.5	X	X
1 6	O	O
1 7	O	O
1 9	X	X
2 0	X	X
2.25	X	X
2.5	X	X

X indicates death
O indicates survival

Table 2 Subarachnoid Heparinized Blood

CC/KG	NORMOTHERMIC GROUP 5	HYPOTHERMIC GROUP 6
1 5	\	
1 8	O	
1 9	\	
2 2	O	O
2 25	O	
2 3	\	
2 3	\	
2 5	O	O
2 5	\	O
2 5	\	\
2 5	\	\
2 5	\	\
2 5	\	\
(3 5)		(O)

\ indicates death
O indicates survival

Table 3 Subdural Whole Blood

CC/KG	NORMOTHERMIC GROUP 7	HYPOTHERMIC GROUP 8
1 1	\	O
1 5	\	
1 8	\	O
1 8		\
2 1	\	
2 25		\
2 4	\	
3	\	

\ indicates death
O indicates survival

SUMMARY

1 In normal dogs there is evidence that hypothermia lowers the intracranial pressure and diminishes brain volume If this were true in dogs subjected to artificial subarachnoid hemorrhage it is possible that hypothermia could increase their chances of survival

2 Mongrel dogs (7 to 8 kg) were subjected to experimental subarachnoid hemorrhage by injecting blood obtained from the femoral artery through a polyethylene catheter previously implanted transcerebrally into the basal subarachnoid space The severity of the hemorrhage was measured in cc of blood/kg of body weight

3 With hypothermia below 25°C most of the animals died irrespective of the size of the hemorrhage

4 Hypothermia of 27° to 30°C did not raise the chances of survival above the normothermic figure

5 Survival was increased by using heparinized blood instead of whole blood in both the normothermic and hypothermic animals

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HYPOTHERMIA AND PIAL CIRCULATION *

L. MURRAY THOMAS, ELISHA S GURDJIAN AND JOHN E WEBSTER

Prompted by the increasing use of hypothermia 12 13 in physiological investigation as well as in the operating room a study of the pial vessels by the cinephotomicrographic means was undertaken The recent literature concerning the metabolic, electroencephalographic, electrocardiographic and blood flow changes during hypothermia has been reviewed This study deals with the dynamic changes of the pial vessels (50 to 200 micra) under the conditions of lowered temperature

METHOD

Eleven experiments were carried out using one mongrel dog and 8 Rhesus monkeys Intravenous sodium pentobarbital was used for anesthesia in all experiments The blood pressure was recorded from the femoral artery with a Statham strain gauge Electrocardiographic and respiratory tracing were obtained In 7 animals an electroencephalogram was recorded The temperatures were measured rectally both with a thermister and a laboratory thermometer Body cooling was accomplished by the use of the cooling blanket and machine manufactured by the Therm O Rite Products Company Five monkeys were cooled to 10°C or lower and one monkey was cooled to 2 8°C with survival

The pial vessels were exposed for study through a trephine opening made in the parietal area In three experiments a glass dipping cone (Leitz) was used between the objective and the pial surface In the remaining 8 experiments a round glass coverslip was inserted between the dura and the piarachnoid through a cruciate incision and the dura was then sutured in a purse string fashion about the periphery of the coverslip On several occasions a coverslip with an etched scale of 0 05 mm was used The vessels could then be studied under conditions similar to the normal closed dura state Photographic resolution and vascular dynamic changes did not appear to be affected by the interchange of these two methods

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Photographic records were obtained on 16 mm Kodachrome type A film using a Kodak Cine Special camera The Leitz Ultropak equipment was used as the camera objective A 534 watt 110 volt, alternating current carbon arc lamp was used for light source A Bausch and Lomb 85/15% beam splitter was used between the camera and the objective to permit continuous observation during photography The vessels were observed at magnifications of 21 to 120 times and were recorded on the film at enlargements of 4 2 to 12 X

RESULTS

Cooling was accomplished quite uniformly at a rate of approximately 1°C each 5 minutes Under the conditions of light pentobarbital anesthesia shivering occurred in several animals requiring the use of additional anesthesia This had the undesirable effect of depressing respiration [5]

The electrocardiogram showed a progressive bradycardia Prolongation of the P R and Q T intervals, elevation of the S T segment and inversion of the T wave as shown by Bering *et al*,[2] were seen in our recordings In addition bizarre T wave changes and disorganized ventricular complexes became apparent at low temperatures (15°C and lower) The abnormalities reversed on warming

The pulse rate decreased in direct proportion to the temperature drop [10] No arrhythmia was noted above 15°C Ventricular fibrillation was a major problem in 2 animals at 9° and 10°C In one animal artificial respiration with 100% oxygen was used Ventricular fibrillation became irreversible and the animal expired In another animal spontaneous respirations continued even at 9°C and in this experiment the fibrillation was of short duration and regular cardiac activity resumed Although the use of hyperventilation has been suggested by Swan *et al*,[11] others [13] have not felt that it is needed Bigelow [3] found that the use of 5% carbon dioxide was useful in prolonged survival in hypothermic dogs Accordingly, we used 5% carbon dioxide artificial respiration on two later occasions where respiratory aid was needed Ventricular fibrillation occurred in one animal (6 5°C) but it returned spontaneously to regular rhythm In the other animal, at 2 8°C, asystole occurred after 10 minutes of a bradycardia of 1 to 2/min The asystole lasted for 15 minutes during which time the blood pressure was 0 The animal then was stimulated with a 150 volt, monopolar 10 millisecond square wave at a frequency of 60/min The blood pressure rose with each stimulation After 10 minutes of stimulation it was 10 to 12/0 mm Hg Stimulation was stopped and the heart continued to beat in a regular fashion As warming progressed the EKG returned to a normal pattern This animal survived without apparent abnormality

The respiratory rate became depressed as the temperature fell Wilson [13] found that the respiratory rate did not become significantly altered until 18 to 20°C *when barbiturate depression was not present* The use of additional pentobarbital to control shivering resulted in marked respiratory depression in several animals

The EEG decreased in amplitude and frequency becoming isoelectric at 18 to 20°C in the monkey [9] and at 20°C in cats [6] In our animals this occurred between 20° and 22°C On warming the EEG activity returned to normal [4]

The blood pressure tends to fall with hypothermia [13] Some authors have felt that the pressure drop was proportional to temperature [8] Others found that

blood pressure had no direct relationship to temperature [10] and, in fact, may not occur in young individuals [11] Our experience is that blood pressure varies considerably in response to hypothermia. In general it tends to fall, but on several occasions it remained essentially stable with temperatures of 20 to 25°C. On warming, the pressure returned to normal but lagged behind the temperature rise.

The pial vessels showed a great constancy of size under our experimental conditions We were not able to confirm the marked reduction in caliber of vessels under 30°C, as seen by Meyer.[8] In our experiments the vessels of 150 to 200 micra in size remained essentially the same although on occasion they would increase in diameter. The smaller vessels (50 to 100 micra) showed a greater variation in size On occasion these vessels would decrease in size and several minutes later at a lower temperature would increase in size. In general, the major branches remain constant while the variation in caliber takes place in anastomotic connections We were able to record small anastomotic vessels completely emptying and filling in relation to the respiratory cycle. Variation in particulate flow through such a vessel would result in an apparent decrease in size. Even at extreme temperatures (2 8°C) we were not able to record major caliber changes The changes that we saw varied from + to —25% and bore no relationship to either temperature or blood pressure. We believe that local as well as general cerebral flow variations and changes in local carbon dioxide concentrations account for the differences observed. In general the cerebral blood flow decreases with hypothermia,[10] but the actual percentage change varies greatly from animal to animal [2] Rosomoff [10] found that the cerebral arterial venous oxygen difference remained the same with decreasing temperatures because of variations in the blood flow Adams *et al* [1] feel that the A/V oxygen difference tends to decrease with lowered temperatures. In our experiments a marked difference in the arterial and venous color differential was noted. The variation was related not to temperature but to major changes in the blood flow particularly controlled by cardiac abnormalities Except for such cardiac changes, no constant difference in the A/V color could be detected. The background becomes more pale as the temperature is lowered (Meyer) [8] This may relate to increased cerebral resistance [2]

In several animals particulate flow was seen in veins at normal temperature. With hypothermia, flow became more evident in the veins and was affected by the pulse and respiratory cycle As the temperature lowered particulate flow could be visualized in the arteries With temperatures of 12°C and below, the arterial cellular material could be seen to surge swiftly forward with each pulse (10 to 20/minute), stop and then move backwards slightly with diastole As the temperature continued lower, the flow became extremely disorganized. At 2 8°C observations and films made during a prolonged period of asystole showed flow in certain areas to have ceased, leaving the field devoid of cellular elements In other areas flow continued in relation to the artificial respiratory cycle As the animal warmed, these vessels again filled with cellular material and a normal flow pattern resulted

SUMMARY

1. With hypothermia the pulse rate falls progressively with temperature
2 The blood pressure and respirations also become depressed with hypothermia but bear no constant relationship to temperature.

3 The EKG exhibits reversible changes with decrease in temperature

4 The EEG likewise evidences decreased amplitude and frequency with hypothermia These changes are reversed with warming but lag behind the improvement in the EKG and pulse

5 The diameter of pial vessels 50 to 200 micra in size tends to remain stable A variation of + to −25% can be seen in these vessels particularly anastomotic branches but these seem related to variations in blood flow and carbon dioxide rather than temperature or blood pressure

6 Arterial particulate flow was observed at low temperatures It was seen to possess a back and forward movement similar to venous flow

7 One monkey was cooled to 2 8°C with survival and apparent normal behavior

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OBSERVATIONS ON THE EFFECTS OF HYPOTHERMIA ON EXPERIMENTAL BRAIN LESIONS*

EDWARD J LASKOWSKI

Empirically the use of hypothermia in neurosurgical practice has become widespread Although its clinical use [1-4] and its numerous physicochemical factors are adequately reviewed [4] controlled studies of the more basic aspects in regard to the effects of hypothermia on brain tissue are still inadequately explored

Cerebral edema constitutes a major neurosurgical problem in view of the enclosure of the brain within rigid anatomical structures Rosomoff has shown that in hypothermia at 25°C the brain volume is reduced 4.1% and

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the available extracerebral intracranial space is increased 31.8%. The brain-to-skull disproportion is thereby reduced to provide for lesser increased intracranial pressures.

The cerebral edema with its relationship to the permeability of the blood-brain barrier and the behavior of the tissue elements has been studied by our group[6] recently. In the course of these studies, it was found possible to make a highly reproducible brain lesion, the dynamics of which could be followed chronologically. The present report constitutes a comparative study of the effects of hypothermia on this standardized lesion.

METHOD

The apparatus used for the production of the cortical lesion consists basically of a stainless steel plate with a 1 × 10 mm. surface and whose stem is fixed into a system of glass tubing in which a mixture of dry ice and acetone is circulated. A microthermistor imbedded below the plate's surface provides for accurate temperature measurement.

Cats, anesthetized with intraperitoneal nembutal, were operated upon under aseptic conditions. Through a 15 mm. trephine opening the pial surface of the left medial suprasylvian gyrus was exposed. The surface of the plate was brought to —50° to —55°C. and then placed in light contact with the cortical surface for 20 seconds. A few drops of Elliotts solution at 37°C. quickly released the frozen plate from the cortical surface. Complete closure of the dura and overlying structures concluded the operation.

For the blood-brain barrier studies, 2 cc. of 10% sodium fluorescein was administered intravenously 45 to 60 minutes prior to sacrifice of the animal. The brains were photographed in ultraviolet light immediately following removal.

After photography, the brains were fixed in preparation for the numerous histologic and histochemic techniques.

To assess the effects of hypothermia, the animals were divided into three groups (Fig. 1). In Group 1, the rectal temperatures were maintained at 37.5°C. during and after the surgical procedure. These were sacrificed by decapitation in groups of 6 animals each at time intervals as follows: immediately, 6, 12, 24, 48 and 72 hours; 7, 30, 60 and 90 days postsurgery. In Group 2, the temperature of the animals was lowered to 28°C. (rectal) before application of the lesion and maintained between 26° to 28°C. for 6 hours before warming and returning to their cages. Of these, groups of 6 animals each were sacrificed at 24, 48 and 72 hours and 7 days. Group 3 is represented by animals in whom rectal temperatures were maintained at 37°C. until 6 hours after production of the cold lesion and were then lowered to and maintained

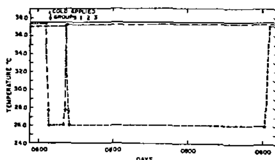


Fig 1 Diagram illustrating temperature course of groups in hypothermia project: Group 1—solid line, Group 2—broken line and Group 3—dashed line.

between 26 0°C and 26 5°C for the remainder of 48 hours Seven of these animals were sacrificed at 48 hours

Hypothermia was induced by covering the body and extremities with ice and warming was accomplished with electric heating pads Rectal temperatures were recorded on a potentiometer at 90 second intervals

RESULTS

Since the observations concerning various stages of our experimental lesion have been already reported in detail, the chronological identical lesions in normothermic and hypothermic animals will be only briefly described and compared

In the normothermic animal maximum involvement was seen at 24 hours The cortical lesion was marked by a sharply demarcated necrotic zone involving the superficial layers of the cortex The underlying white matter presented the picture commonly associated with edema In myelin stains there was a pallor due to a separation of the myelinated fibers and no disintegration of the myelin could be seen Sections stained with PAS revealed a diffuse PAS positive staining of the interstitial spaces, particularly in the proximity of the blood vessels There was also an intense PAS positive staining of astrocytic cytoplasm and expansions The histochemical analysis indicated the glycoprotein nature of the PAS positive substances in the area of edema

Cajal gold chloride stain revealed the astrocytes to be markedly enlarged and usually elongated in an axis perpendicular to the course of the nerve fibers The expansions appeared thickened and granular At times the intracytoplasmic fibrils could be seen distinctly in the granular cytoplasmic matrix of the astrocytes Yet, in astrocytes which exhibited moniliform changes the intracytoplasmic fibrils were not recognizable Oligodendroglia showed no appreciable changes and, particularly, acute swelling of these cells was never observed No microglial response was noted

Study of the blood brain barrier revealed a cone shaped area of fluorescence extending through the white matter toward the base of the gyrus Small nonfluorescent areas, scattered through this fluorescence were present mostly in the cortex

At 24 hours the hypothermic animals in Group 2 showed an area of fluorescence extending into the white matter just below the cortex with small scattered areas of nonfluorescence confined to the cortical area alone The area of PAS positive staining was definitely less in extent than that in the normothermic animals The gold chloride stain revealed less marked distension of the astrocytes in the hypothermic group

The 48 hour normothermic animal showed a reduction in size of the fluorescent area whereas the hypothermic animal showed an increase in the area of fluorescence as compared to the 24 hour lesion This was the maximal area of fluorescence in the Group 2 animals, nevertheless, it still appeared considerably smaller than the maximal area of the 24 hour Group 1 animals Similar to the fluorescence, the PAS positive staining was more extensive in the 48 hour than the 24 hour hypothermic animals and resembled the 24 hour normothermic group

At 72 hours, the fading of fluorescence was apparent in both Groups 1 and 2, but was somewhat more conspicuous in the hypothermic animal Similarly the PAS positive staining though still conspicuous, was greatly diminished

in intensity in both groups with the hypothermic animals showing less staining than their normothermic counterparts The reactive astrocytes appeared to be the same size in both groups

At 7 days, no differences in the permeability of the blood brain barrier and appearance of the tissue elements could be ascertained. The areas of fluorescence were both limited to the cortex. Histologic evidence of edema was absent PAS positive staining was confined to the blood vessel walls The astrocytes were markedly hypertrophic. No animals were sacrificed beyond 7 days in Group 2.

In Group 3, the area of uniform fluorescence was limited to the superficial portion of the cortex. PAS positive staining in the area of edema was definitely less pronounced than in Groups 1 and 2 However, the widening of the white matter in the gyrus was similar to the 18 hour stage of the other groups

In recovered animals of Groups 1 and 2, no neurological deficits were demonstrable.

CONCLUSION

The main points of interest derived from this study are as follows:

1. After 24 hours, the animals under 6 hours of hypothermia showed a smaller area of fluorescence, less pronounced PAS positive staining and less intense astrocytic response than normothermic control animals

2 At 48 hours, in the hypothermic group there seems to be a certain "rebound" phenomenon, manifested by an increased permeability of the blood brain barrier in comparison to the 24 hour animals. However, this is still substantially less than the maximal fluorescence seen in the 24 hour normothermic animals The astrocytic response and PAS positive staining are comparable in both groups

3 After one week, the area of fluorescence, the PAS positive staining and the degrees of astroglial response in both groups are indistinguishable.

The close relationship between edema and permeability of the blood brain barrier and PAS positive reactions in the white matter have been previously established In view of this, a measure of benefit provided by the reduction in edema during the first 18 hours following the use of hypothermia in intra cranial neurosurgical procedures might be expected There is no evidence, however, that hypothermia offers any protection against the ultimate post traumatic astroglial proliferation

The therapeutic value of prolonged hypothermia for severe brain injury is suggested by the observations in Group 3 animals These animals showed a marked reduction in the area of fluorescence in contrast to animals of Groups 1 and 2 at 48 hours. The extent and intensity of the PAS positive staining were reduced and there was less pronounced astrocytic response However, the observations in this group cannot be regarded as conclusive in view of the limited number of animals studied to date The extended studies are in progress and will be reported

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APPLICATION OF POSITRON EMITTING ZIRCONIUM 89 FOR POTENTIAL USE IN BRAIN TUMOR LOCALIZATION*

JOHN MEALEY, JR.

Over 2,400 patients suspected of harboring brain tumors or other intracranial lesions have undergone external isotopic encephalometry with the automatic scanning apparatus developed by Brownell, Aronow, and Sweet.[1,2] This apparatus detects and graphically records abnormal isotope concentrations by counting the coincidences of annihilation gamma radiation given off after injection of positron emitting isotopes.

In the vast majority of these studies Arsenic 74 has been used with a continued detection of 75% of all types of brain tumors.[3,4] Copper 64 has also been employed clinically and was found useful in the detection of 55% of a random series of brain tumors.[4]

Zirconium 89 is one of several other positron emitting isotopes which have been studied with regard to their applicability and usefulness for external localization of intracranial lesions by the coincidence scanning technique. The stable element Zirconium is one of the rarer heavy metals with an atomic weight of 99.22. As Zirconium oxide, this element has been used in dermatology and in contrast radiography in place of barium. Zirconium 89 emits positrons and gamma rays of about 0.9 Mev energy. It has a convenient halflife of some 80 hours.

A carrier free solution of Zirconium 89 is obtained by means of deuteron bombardment of a Yttrium oxide target in the cyclotron at Massachusetts Institute of Technology. The Zirconium 89 activity is separated from the dissolved target substance by using an ion exchange resin and is administered intravenously in a sterilized filtered citrate complex of pH 6. No ill effects were noted in toxicity studies in mice receiving proportionally massive doses. No adverse reactions have occurred after injection in humans.

Distribution studies in mice were carried out after intraperitoneal injection of Zirconium 89. Skull, kidney, heart, liver, and muscle, in order of decreasing concentration, accumulated a substantially larger amount of the tracer substance than did brain during the first 24 hours after injection.

The distribution of Zirconium 89 in man after intravenous injection was also determined in blood, urine, and biopsy samples of muscle, bone, normal brain, cerebrospinal fluid, and brain tumor.[5] It was found that Zirconium 89 disappears from plasma slowly because of the formation of metal complexes with the protein fractions. Only about 1% of the injected dose was recovered per day from the urine. This indicates that the effective biologic halflife is virtually equal to the 80 hour physical halflife. In contrast to that found in mice, Zirconium 89 concentration in human biopsy samples was proportionally much less in bone and far greater in muscle. Concentration in brain remained low, and tumor brain ratios of 6:1 were obtained.

A group of selected patients with previously verified intracranial lesions were scanned with Zirconium 89. A dose of 0.3 mc of Zirconium 89/70 kg body weight in adults provides satisfactory graphic recordings. Because of

* From the Neurosurgical Service, Massachusetts General Hospital, Boston. Supported in part under Contract AT(30-1)-1242(A7) by the United States Atomic Energy Commission.

the lower total dose administered and the shorter physical halflife, the whole body radiation absorbed after Zirconium 89 injection, despite its slow excretion, is less than that absorbed after the usual dose of Arsenic-74.

In general, the configuration of the normal scan or positrocephalogram obtained after injection of Zirconium 89 as depicted in Figure 1 is not unlike those produced with Arsenic-74. In contrast to the scans with Copper-64, the peripheral scalp and skull outline is less well defined with Zirconium-89. Concentration or density in the facial, temporal, and occipital areas is pronounced because of the great affinity of Zirconium 89 for muscle. This feature would empirically seem to rule out detection of basilar and posterior fossa tumors with this isotope. Serial scans on 2 patients with proven metastatic cerebellar tumors have not been diagnostic.

Zirconium 89 scans in several patients with surgically verified gliomas suggest a potential usefulness of this isotope in preoperative localization of this group of intracranial lesions. Figure 2 shows the positrocephalogram obtained after injection of Zirconium 89 in a 35 year old white male who one year earlier underwent a left frontal craniotomy and subtotal removal of a glioblastoma multiforme of the frontal lobe. He received a course of postoperative X-ray therapy. The denser stamp markings in the frontal area indicate a higher concentration of the isotope in the tumor as contrasted with other more normal areas. Figure 3 illustrates a sagittally projected scan from this patient, and it can be seen that the area of higher density in the tumor is largely to the left of midline.

Zirconium 89 has concentrated also in more slowly progressing gliomas. Figure 4 shows an asymmetrogammagram with left sided unbalance in a 40 year old white male with a Grade II astrocytoma in the left parietooccipital area. This tumor was verified 4 years earlier by craniotomy and biopsy. This patient also received postoperative X-ray therapy. The straight line markings seen in the parietooccipital zone correspond to the left sided locus of the tumor. Similar but curved stamp markings would indicate a right sided location of increased concentration. The positrocephalogram in this patient, recorded simultaneously with the unbalance scan, also indicated a parieto-occipital concentration.

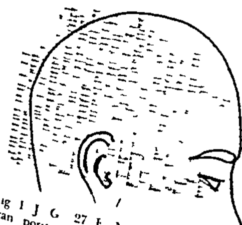


Fig. 1. J. G. 27 F. Normal Zirconium 89 scan positrocephalogram.

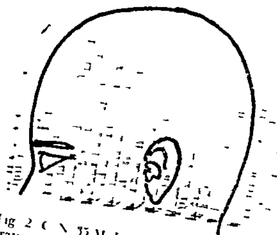


Fig. 2. C. N. 35 M. Increased frontal concentration of Zirconium-89 in a lateral scan of a patient with glioblastoma multiforme.

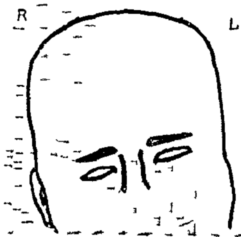


Fig 3 C N 35 M Sagittal scan in the same patient as in Figure 2 Area of higher density on the left side corresponds to the tumor

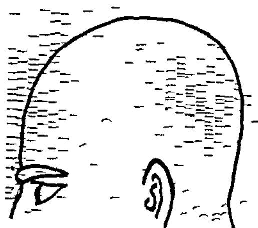


Fig 4 J W 40 M Asymmetrogammagram showing left-sided unbalance or concentration in a patient with a left parieto occipital astrocytoma grade II

An opportunity has arisen on two occasions to compare Zirconium 89 scans with those obtained with Arsenic 74 within a suitably short interval of one another in patients with recurrent meningiomas who were subsequently reexplored In one individual with a recurrence limited to the orbit the preoperative scans were virtually identical with the two different isotopes Both showed increased density over the affected orbit in the lateral scans However in the other individual a woman with a recurrent parasagittal parietooccipital meningioma the localization with Zirconium 89 was far less striking than that demonstrated by the Arsenic 74

Because of the prolonged intravascular phase after injection of Zirconium 89 a possible use for this tracer in the detection of intracranial hemorrhage has been considered Biopsy data obtained from a patient with a subdural hematoma gave a 3 1 ratio between dark, liquefied hematoma and normal brain Scans of two patients with traumatic cerebral lacerations however were unremarkable 1 and 3 weeks after the original injury No opportunity has yet arisen to attempt localization of a surface or intracerebral hematoma by scanning with Zirconium 89

SUMMARY AND CONCLUSIONS

The development and certain biological features of a carrier free solution of positron emitting Zirconium 89 applicable for external coincidence scanning of the head is described

Preliminary scans of selected patients with known lesions suggest a potential usefulness of this isotope in the localization of supratentorial tumors particularly in the glioma group

It remains to be determined whether scanning with Zirconium 89 will provide 1) any significant advantage over the experience with Arsenic 74 and Copper 64 in the clinical detection of these tumors preoperatively and 2) any clinical usefulness in the diagnosis of intracranial hematomas

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KINKS AND COILS OF THE CERVICAL CAROTID ARTERY*

H Harvey Gass

The usual anatomical course of the cervical portion of the internal carotid artery is a straight path from its origin at the bifurcation of the common carotid artery to the base of the skull. Although minor gentle curves may be present in this portion of its course, sharp angulations or sinuous tortuosities would be considered deviations from normal anatomy. Such variations could be of importance in selected instances of carotid artery insufficiency not otherwise explained. Kinks and coils of the cervical portion of the internal carotid artery have received scant attention from neurologists and neurosurgeons. Otolaryngologists, however, have become familiar with this entity because of the occasional fatal hemorrhage during tonsillectomy due to surgical invasion of one of these redundant loops protruding into the lateral pharynx. Their interest in the problem has stimulated sufficient investigation to establish the fact that such redundant loops of artery at the level of the pharynx are not rare. The present study reporting on a number of anomalies of this type encountered unexpectedly during angiography by a single worker during a short time stresses the frequency of this finding.

Anatomical deviations of the internal carotid artery other than redundant looping at this particular pharyngeal level, however, have been ted. Since this report was submitted, which is based on 7 instances of these anomalies, we have encountered an additional 10 occurrences. The 7 reported here, however, are typical of the entire group. They include tortuosity and coiling of the artery, sharp angulation without coiling, sharp angulation with coiling and changes in the caliber of the artery in conjunction these anatomical deviations. Although these lesions occurred most commonly at the level of the atlas adjacent to the lateral pharynx, some occurred at lower levels as well (Fig 1 & 2).

There is reason to think that the cause of these anomalies is embryologic and not the result of some pathologic process such as arteriosclerosis, hypertension, inflammation or accidental kinking which might occur during life time. The development of the internal carotid artery in the embryo involves the absorption of several primitive aortic arches while others destined to become mature vessels remain. During this process, the vascular channels which ultimately persist to become the internal carotid artery do take a winding course. As the embryonic vasculature matures this winding course straightens out. It is possible that a differential rate of growth between the

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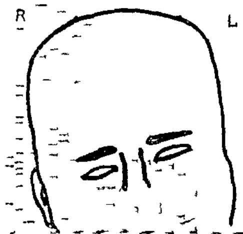


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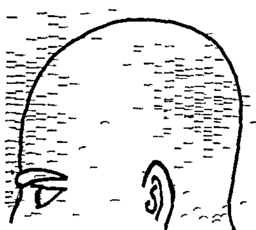


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The usual anatomical course of the cervical portion of the internal carotid artery is a straight path from its origin at the bifurcation of the common carotid artery to the base of the skull Although minor gentle curves may be present in this portion of its course sharp angulations or sinuous tortuosities would be considered deviations from normal anatomy Such variations could be of importance in selected instances of carotid artery insufficiency not otherwise explained Kinks and coils of the cervical portion of the internal carotid artery have received scant attention from neurologists and neurosurgeons Otolaryngologists however have become familiar with this entity because of the occasional fatal hemorrhage during tonsillectomy due to surgical invasion of one of these redundant loops protruding into the lateral pharynx Their interest in the problem has stimulated sufficient investigation to establish the fact that such redundant loops of artery at the level of the pharynx are not rare The present study reporting on a number of anomalies of this type encountered unexpectedly during angiography by a single worker during a short time stresses the frequency of this finding

Anatomical deviations of the internal carotid artery other than the redundant looping at this particular pharyngeal level however have been noted Since this report was submitted which is based on 7 instances of these anomalies we have encountered an additional 10 occurrences The 7 reported here however are typical of the entire group They include tortuosity and coiling of the artery sharp angulation without coiling sharp angulation with coiling and changes in the caliber of the artery in conjuction with these anatomical deviations Although these lesions occurred most commonly at the level of the atlas adjacent to the laternal pharynx some occurred at lower levels as well (Fig 1 & 2)

There is reason to think that the cause of these anomalies is embryologic and not the result of some pathologic process such as arteriosclerosis hypertension inflammation or accidental kinking which might occur during life time The development of the internal carotid artery in the embryo involves the absorption of several primitive aortic arches while others destined to become mature vessels remain During this process the vascular channels which ultimately persist to become the internal carotid artery do take a winding course As the embryonic vasculature matures this winding course straightens out It is possible that a differential rate of growth between the

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Fig 1 Coiling of the left internal carotid artery at the level of the atlas in a 55 year old male with three episodes of nausea and syncope Left carotid compression not tolerated because of impending syncope



Fig 2 Marked kinking of the internal carotid artery in a 61 year old female who had had one episode of contralateral hemiparesis and one episode of syncope with post syncopal amnesia and confusion

arterial and skeletal systems in these patients may also be an etiologic factor Whether or not congenital bands binding down the carotid artery at certain levels in the neck play a role remains to be seen In a report by Boldrey, *et al*[1] a case is presented in which adhesions thought by them to be inflammatory bound down the artery to the lateral mass of the atlas It was felt that such adhesions could be responsible for carotid artery thrombosis at that level and also for the tortuous duplication which they also noted commonly present there It is however also possible that such adhesions or bands may be congenital rather than inflammatory There is further supporting evidence in favor of an embryologic etiology in 2 of the author's patients who had other vascular anomalies involving the same carotid tree In one, carotid kinking in the neck was present as well as an aneurysm arising from the homolateral carotid artery intracranially (Fig 3) The other was a 2 year old child recently seen who had both tortuosity of the carotid artery in the neck and a presistent primitive trigeminal artery connecting that carotid artery intracranially to the basilar artery (Fig 4)

The clinical importance of this presentation, however, has to do with the role that these anomalies may play in the production of carotid artery insufficiency Of the 7 instances forming the basis for this report, 2 occurred in one patient they being bilateral instances of this anomaly In the group of 6 patients 5 had clinical manifestations which might have occurred as a result of carotid artery insufficiency In 1 of these, all normotensive patients, a sudden onset of contralateral hemiparesis or unexplained sudden loss of consciousness occurred without subarachnoid hemorrhage and without other



Fig 3 Double kinking at the level of the atlas in a 31 year old female with spontaneous subarachnoid hemorrhage Berry aneurysm arising from the intracranial carotid artery is also present



Fig 4 Tortuosity of the cervical internal carotid artery at the level of the atlas in a 2 year old boy with repeated transient contralateral hemiparesis who also had a persistent primitive trigeminal artery (carotid basilar communication)

evidence of cerebral disease Three of this group had more than a single such episode and good recovery followed each attack The fourth patient of this group had bilateral coiled anomalies (Fig 5) He was hospitalized following a single episode of sudden loss of consciousness No other explanation was found Right carotid compression in this patient readily produced faintness with impending syncope In a fifth patient the sole presenting complaint was a bruit heard over the homolateral mastoid area which disappeared following the angiogram Such bruits have been reported in conjuction with partial carotid artery occlusion In the sixth patient the coiling was an incidental finding in a woman with a ruptured homolateral berry aneurysm arising from the intracranial portion of the carotid artery

Kinking of the arteries without coiling sufficient to narrow the lumen at the point of angulation was evident in the angiograms in three instances and in one other instance with associated coiling Multiple projections at different obliquities may be necessary however to demonstrate such kinking to the best advantage This effort was not always made

In one patient a 38 year old male with recurrent left hemiparesis and/or left hemisensory syndrome and subsequent improvement after each episode, surgical exploration was done to see if the marked kinking which was felt to be responsible could be corrected (Fig 6) At operation † the kinking



Fig 5 Bilateral coiling at the level of the atlas in a 66 year old male with a single episode of syncope and post syncopal confusion Right carotid compression not tolerated

† Surgery performed by Dr Homer Smathers

was well demonstrated and the artery proximal to the kink was found to be strikingly distended and markedly narrowed distal to it The redundant coil was excised and an end to end anastomosis done Because of the different calibers of the two ends technical difficulties were encountered Nevertheless immediate satisfactory restoration of circulation was established following the



Fig 6 Marked kinking in a 38 year old male with recurrent contralateral neurological episodes Correction by excision of redundant artery with end to end anastomosis although initially successful eventually failed because of thrombosis at operative site which fortunately was asymptomatic

anastomosis A repeat angiogram 3 months later however showed complete obstruction at the site of anastomosis which fortunately had been tolerated by the patient without incident No further opportunity has yet presented itself for further surgical efforts It is possible however that if an anastomosis could be made with ends of even caliber after resection of the redundant loop or if restricting fibrous bands were present which might be lysed or if the course of the artery might be altered to reduce the redundancy by rerouting it insufficiency if it existed might be corrected It might be possible for example to cut the digastric tendon and resuture it beneath the internal carotid artery thereby lengthening the course of the artery and reducing its kinks or the artery might be tacked up to the sternocleidomastoid muscle as Riser *et al*[2] have done They reported a case of probable carotid artery insufficiency with vertiginous crises consisting of attacks of sudden vertigo headache sweating and nausea occurring several times daily or weekly due they believed to redundant coiling of the artery in the neck They treated their patient by tacking the artery up to the underside of the sternocleidomastoid muscle to unroll the coiling During a 4½ month postoperative followup except for the immediate postoperative period no further attacks occurred

SUMMARY

It is the purpose of this report to draw attention to these anomalies in order to stimulate accumulation of clinical data so that their role in the production of carotid artery insufficiency may be established It is not the authors intent to imply that such anomalies are invariably symptomatic but on the other hand to suggest the possibility that in many instances under certain circumstances of head position hypotension stress or other crises certain ones of them may produce insufficiency If these could be identified surgical correction may be possible

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CORTICAL VEINS OF THE HUMAN BRAIN*

D. M. PERESE

The study of the cortical veins of the human brain was undertaken to gather information which would be of practical value to a neurological surgeon.

The excellent reviews[2,3] on the embryonic development of the human cranial venous system lack the information most needed in the operating room.

METHOD

The problem of the distribution, points of drainage and anastomosis between the surface vessels of the brain was investigated in the autopsy material. The brains of 100 patients aged 15 to 78 years with no involvement of the central nervous system were used in this study. The venous system of the brain was injected through catheters in the superior longitudinal and straight sinuses, and both jugular veins. The material injected was neoprene latex solution containing a suspension of ordinary painter's lead. The brains were removed 3 to 4 hours later by the author and the communicating veins between the brain, dura and bone carefully recorded. These brains were x-rayed in the cranium before removal as well as after complete fixation. Each brain was removed with the dura intact over the entire cerebrum and most of the cerebellum. The brains were suspended in 10% aqueous formalin.

RESULTS

The analysis of the study indicated the following topographic pattern: veins of the lateral surface of the cerebrum, veins of the medial surface of the cerebrum, veins of the inferior surface of the cerebrum, and cerebellar veins.

The veins on the lateral surface of the cerebrum consisted of frontal and parietal veins draining into the superior longitudinal sinus and temporal and occipital veins draining into the lateral sinus. In addition there was a third group serving as an auxiliary channel between the superior longitudinal sinus and the lateral sinus.

The important veins in the frontal group consisted of frontopolar, inferior, middle and superior frontal veins (Fig. 1). The frontopolar veins consisting of 2 to 6 branches on each side drained the anterior half of the gyrus rectus, the orbital gyri and the pars orbitales of the inferior frontal gyrus. The frontopolar vein had a free course from 0.6 cm to 1.8 cm in the subdural space. The visualization of these vessels before the complete mobilization of the frontal pole helps to avoid cumbersome bleeding. The other frontal veins entered the dura a considerable distance, 1.5 to 3.5 cm, off the midline and had a long free course, 3 cm, in the subdural space whereas the parietal veins pierced the pia quite near the midline traveling in the subdural space for a short distance, 0.2 cm to 0.5 cm, before attaching to the under surface of the dura. There were no veins running from the occipital lobe to the superior sagittal sinus. They crossed the parietal lobe before entering the sinus.

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* From the Roswell Park Memorial Institute, Buffalo, N. Y.



Fig 1 White arrow shows the frontopolar vein draining into the superior longitudinal sinus and into the angular vein through the emissary vein Black arrows indicate the inferior middle and superior frontal veins

The temporal and occipital veins are made up of the so called vein of Labbe which consists of two or more branches originating in the Sylvian fissure These vessels joined the lateral occipital veins at the pre occipital notch and entered the tentorium to drain into the lateral sinus

The anastomatic veins of the middle cerebral group were capable of carrying the venous blood from the frontal, parietal and part of the occipital lobe as well as from the temporal lobe The middle cerebral veins followed the Sylvian fissure, communicated with the basal vein of Rosenthal and with deep veins draining the insula, and emptied into the sphenoparietal, cavernous and middle meningeal sinuses

In general no bridging veins were found along the inferior lateral edge of the frontal lobe and the entire lateral surface of the occipital lobe

The inferior surfaces of the frontal lobes drain into the superior sagittal sinus the middle cerebral veins and the basal vein of Rosenthal The inferior surface of the temporal lobe drains into the lateral sinus and the basal vein (Fig 2) The inferior surface of the occipital lobe drains into the lateral sinus and the vena magna of Galen The pattern described above was present in more than 70% of the brains studied

The medial surface of the hemisphere has two sets of veins anastomatic veins and the anterior and posterior cerebral veins (Fig 3) The anastomatic veins are between the inferior and the superior longitudinal sinus, #6 to 8 and start above the corpus callosum These anastomatic veins enter the superior longitudinal sinus within a millimeter or two of the midline During an attempt to isolate the sinus in its length the convexity as well as the short medial surface anastomatic veins should be considered The veins around the corpus callosum are not continuous but consist of an anterior cerebral branch in front of the genu of the corpus callosum which joins the basal vein and a posterior cerebral branch which receives one or two branches from the medial surface of the parietal lobe and circles the splenium of the corpus collosum before entering the vena magna

Cerebellar veins can be grouped under three categories—superior cerebellar veins great anterior cerebellar vein, and posterior cerebellar veins The superior cerebellar veins consist of 2 to 4 veins in the midline of the superior surface of the cerebellum and drain upward into the great vein of Galen Additional branches join them from brachium conjunctivum and the entire



Fig 2 Arrow shows the middle cerebral vein joining the basal and middle meningeal veins



Fig 3 Anastomatic veins of the medial surface of the hemispheres are numbered from front to back consecutively (1 to 6) Arrow shows the anterior cerebral vein

superior surface of the cerebellum Occasionally the superior cerebellar vein is a large single trunk running under the sinus rectus (Fig 4) On reaching the posterior cerebellar notch it turns downward running on either side of the vermis During a surgical attack requiring the exposure of the inferior colliculus the superior cerebellar veins can be cauterized and divided either partially or totally without serious sequelae provided that special attention is paid to the vein of Galen

The great anterior cerebellar vein (also called petrosal vein) receives branches from the horizontal sulcus between brachium pontis and the anterior cerebellar lobe of the lateral hemisphere from the pontine venous plexus and the inferior temporal surface of the corresponding half of the hemisphere All these branches join together at the base of the fifth nerve forming one or two major branches and run into the superior petrosal sinus behind the 5th nerve The portion of the vein behind the nerve is visible to the surgeon during surgical exposure[1] However the presence of the hidden branches anterior to the 5th nerve is not very well known This represents a concealed trap for the surgeon who confidently approaches the 5th nerve following the division of the petrosal vein



Fig 4 Arrow shows the superior cerebellar vein starting above the cerebellar tonsils running under the straight sinus and entering the vein of Galen above the junction of the internal cerebral and basal veins

The posterior median veins run along the vermis to the straight sinus directly exposing a short, 0 2 cm to 0 5 cm, free portion between the pia and the sinus The posterior lateral cerebellar veins number two to four on each side In contrast to the median veins the lateral veins roll over the superior semilunar lobe and run a short distance, 0 5 cm to 1 5 cm, on the superior surface of the hemisphere before rising to enter the tentorium to drain into the straight or the lateral sinus Their free portion between the pia and the dura is relatively long, 1 0 cm to 2 0 cm The presence of the posterior lateral cerebellar veins on the superior surface of the hemisphere is a definite hazard for the inexperienced surgeon All cerebellar veins have rich networks of anastomosis

SUMMARY

Injection of the venous system of the human brain was carried out in 100 autopsies using neoprene latex combined with lead tetraoxide

Each brain was studied to determine the general pattern of the cortical veins and their branches draining away from the gray matter The results of this study indicate that, in any surgical attack directed toward a lobe of the cerebrum and cerebellum, the location and course of the veins bridging that region of the brain with the dura or cranium can be anticipated with 70% accuracy

Variations from the general pattern described in the text were found to be less than 30% including all surface veins

The tendency to drain away exclusively the area supplied by an individual artery (e g middle cerebral) was a constant finding

The significance of these findings in regard to surgical procedures directed to the brain is discussed

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AN EXPLORATION OF FACTORS INVOLVED IN THE PRODUCTION AND PREVENTION OF PARAPLEGIA FOLLOWING INTRAAORTIC UROKON INJECTION *

EDWARD M LANCE, DUNCAN A KILLEN, AND GUY OWENS

Reports of paraplegia following the employment of urokon 70% (sodium acetrizoate) in abdominal aortography prompted this attempt to define the mechanisms involved in the production and prevention of spinal cord injury following intraaortic urokon injection

* From the S R Light Laboratory for Surgical Research Department of Surgery and the Department of Anatomy Vanderbilt University School of Medicine Nashville Supported in part by the Research and Development Division Office of The Surgeon General Department of the Army, under contract No DA 49 007 MD 697

METHOD

Abdominal aortic and lumbar arterial injections of urokon (Mallinckrodt) (35% or 70% wt./vol.) were effected in 93 mongrel dogs using the standardized technique previously described.[1]

In 4 other animals the toxic effect of direct topical application of urokon 70% to the lumbar spinal cord was assessed.

Simultaneous bipolar electroencephalograms and electromyelograms were obtained for the purpose of evaluation of the central nervous system response to injury in certain of the aortic injection group previously immobilized with succinyl choline.

The standard method of aortic injection of urokon 70% was combined with the following maneuvers in an attempt to modify the paraplegic reaction observed in the control group:

Lumbar Artery Division. Injections were performed following recovery from division of: 1) all lumbar arteries proximal to the origin of the left renal artery; 2) all lumbar arteries distal to the origin of the left renal artery; and 3) all lumbar arteries as well as the middle sacral artery.

Spinal Anesthesia. Injections were performed after high level spinal anesthesia had been obtained by the instillation of 30 mg. of procaine in the cisterna magna.

Hypothermia. The body temperature was cooled to 27° to 29°C. before injection.

Antecedent Medication. Sixty seconds prior to injection of urokon, aortic perfusion (using the standardized urokon aortic injection technique) of the following materials was effected: 1) levophed, 2 u/kg. diluted with normal saline to 15 cc.; 2) procaine, 10 mg./kg. diluted with normal saline to 15 cc.; and 3) 20% glucose, 5 cc./kg.

All animals that survived urokon insult and demonstrated hind limb motor deficit were subjected to weekly neurological evaluation until death or sacrifice (up to 3 months). No animal was sacrificed until a minimum followup period of 3 weeks had been obtained.

Histologic examination of hematoxylin and eosin stained sections of representative spinal cords supplemented the clinical observations.

RESULTS

Of the 97 animals subjected to urokon insult, 73 survived long enough to permit adequate neurological evaluation. Figure 1 illustrates the type, frequency and severity of neurological deficit in the various control groups.

The injection of urokon triggered an immediate and characteristic central nervous system response consisting of the following events: extensor rigidity of the hind limbs progressing to frank myoclonic convulsive movements which were in turn succeeded by transient hyperreflexia.

Electromyelographic recordings taken from the region of the lumbar cord enlargement demonstrated drastic electrical changes occurring immediately upon aortic injection of urokon (Fig. 2) Four per second slow waves appeared first and were replaced over a period of 40 seconds by slow spike forms. At the end of about 2 minutes the pre-injection pattern returned in some cases. Simultaneous recordings of cerebral electrical activity were normal.

Lumbar subarachnoid injection of 5 cc. of urokon 70% in 2 animals

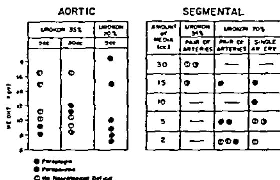


Fig 1 Neurological response of animals of different weights to intra aortic injection of 35% or 70% urokon Neurological response to lumbar arterial injection of varying amounts of 35% or 70% urokon Each hind limb individually illustrated

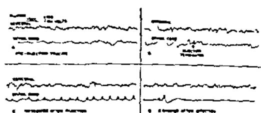


Fig 2 Simultaneous bipolar electroencephalographic and electromyelographic tracings recorded during intra aortic injection of urokon in an animal under succinyl choline immobilization Note the immediate post injection appearance of abnormal spinal cord electrical activity This animal was paraplegic following dissipation of the succinyl choline effect

resulted in respiratory arrest in a matter of minutes Postmortem roentgenograms revealed that urokon had been inadvertently disseminated into the basal cisterns Two other animals were subjected to topical application of 1 cc of urokon 70% to the lumbar cord under direct vision None of these animals exhibited the characteristic immediate motor response previously described and the two surviving animals showed no evidence of hind limb motor deficit

The results derived from the attempts to modify the neurological damage resulting from intra aortic injection of urokon 70% are shown in Figure 3

Intraaortic injection of urokon 70% was performed in 65 animals, and of this group 20 (31%) failed to survive for adequate neurological evaluation No factor other than the injection of urokon could be implicated in the early demise of these animals Of the 45 animals surviving to permit initial neurological evaluation only 24 lived beyond one week

Only one of 40 animals rendered paraplegic during the course of this study showed any return of motor function Intermediate degrees of neurological deficit occurred in eleven other animals of which 6 demonstrated evidence of return of motor function

Microscopic lesions were limited to the region of the lumbar enlargement and sacral cord There was necrosis and dissolution of the central gray

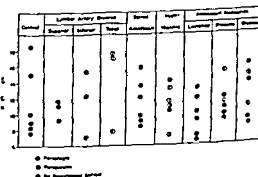


Fig 3 The neurological deficit shown by those animals receiving intra aortic injection of 15 cc of urokon 70% The protective effect of lumbar artery ligation hypothermia and antecedent aortic injection of procaine or glucose can be seen Animal weights are plotted on the ordinate

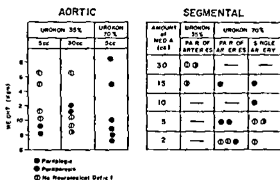


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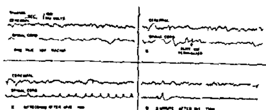


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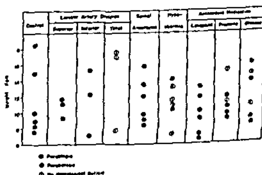


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matter, although preservation of the adjacent myelinated long column structures was noted. In no instance could vascular thrombosis be demonstrated.

DISCUSSION

The neurotoxic potential of urokon 70% is obvious. This toxicity seems to be related more to concentration than to total dose injected, as evidenced by the failure of the same total amount when given as a 35% solution to consistently produce neurologic damage.

Arterial access to the spinal cord seems essential for the production of the neuronal damage leading to paraplegia, as topical application of urokon, while lethal to the exposed brain stem nuclei, was innocuous to the white matter ensheathed spinal cord neurons.

The apparent affinity of urokon 70% for central nervous system tissue is demonstrated by the paraplegic response to small amounts injected into a limited area such as is supplied by a pair of lumbar arteries. Conversely, total lumbar arterial interruption consistently affords protection from paraplegia.

Hypothermia and, to a lesser degree, prior aortic injection of glucose or procaine ameliorated the paraplegic response to intra-aortic injection of urokon. Kenan *et al*[2] have also reported that these factors afford protection from urokon central nervous system damage.

The implication of this study is that the mechanism of urokon injury to the central nervous system is a direct cytotoxic effect rather than a secondary phenomenon resulting from arterial spasm, vascular thrombosis or capillary endothelial alterations. This hypothesis is based on the following evidence: (a) the myoclonic seizure simulates the central nervous system response to known nerve cell toxins, (b) electromyelographic and clinical evidence of abnormal central nervous system activity so promptly follows arterial dissemination of the urokon that tissue anoxia as the primary cause of nerve cell injury is precluded, (c) no microscopic evidence of vascular wall damage or thrombosis in areas of massive nerve cell necrosis was observed, and (d) protection of the neuron from destruction was afforded by those maneuvers which presumably modified cellular metabolism.

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AORTIC

SEGMENTAL

AMOUNT of MEDIA (cc)	UROKON 35% PAIR OF ARTERIES	UROKON 70% PAIR OF ARTERIES	UROKON 70% SINGLE ARTERY
30	◐◐	—	—
5	◐	●	●
0	—	—	●
5	—	●●	◐◐
2	—	◐◐●	◐

Neurological Defect

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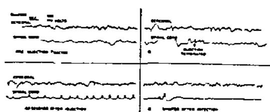


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Control

Spinal Anesthesia

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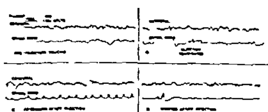
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nly one of 10 animals rendered paraplegic during the course of this ly showed any return of motor function Intermediate degrees of neurolog deficit occurred in eleven other animals of which 6 demonstrated evidence eturn of motor function

licroscopic lesions were limited to the region of the lumbar enlargement sacral cord There was necrosis and dissolution of the central gray

3 The neurological deficit shown by animals receiving intra aortic injection cc of urokon 70% The protective ef of lumbar artery ligation hypothermia antecedent aortic injection of procaine lucose can be seen Animal weights are ed on the ordinate

matter, although preservation of the adjacent myelinated long column structures was noted. In no instance could vascular thrombosis be demonstrated.

DISCUSSION

The neurotoxic potential of urokon 70% is obvious. This toxicity seems to be related more to concentration than to total dose injected, as evidenced by the failure of the same total amount when given as a 35% solution to consistently produce neurologic damage.

Arterial access to the spinal cord seems essential for the production of the neuronal damage leading to paraplegia, as topical application of urokon, while lethal to the exposed brain stem nuclei, was innocuous to the white matter ensheathed spinal cord neurons.

The apparent affinity of urokon 70% for central nervous system tissue is demonstrated by the paraplegic response to small amounts injected into a limited area such as is supplied by a pair of lumbar arteries. Conversely, total lumbar arterial interruption consistently affords protection from paraplegia.

Hypothermia and, to a lesser degree, prior aortic injection of glucose or procaine ameliorated the paraplegic response to intra-aortic injection of urokon. Kenan *et al*[2] have also reported that these factors afford protection from urokon central nervous system damage.

The implication of this study is that the mechanism of urokon injury to the central nervous system is a direct cytotoxic effect rather than a secondary phenomenon resulting from arterial spasm, vascular thrombosis or capillary endothelial alterations. This hypothesis is based on the following evidence: (a) the myoclonic seizure simulates the central nervous system response to known nerve cell toxins, (b) electromyelographic and clinical evidence of abnormal central nervous system activity so promptly follows arterial dissemination of the urokon that tissue anoxia as the primary cause of nerve cell injury is precluded, (c) no microscopic evidence of vascular wall damage or thrombosis in areas of massive nerve cell necrosis was observed, and (d) protection of the neuron from destruction was afforded by those maneuvers which presumably modified cellular metabolism.

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COMPLICATIONS WITH HYPAQUE CEREBRAL ARTERIOGRAPHY*

JAMES S BROWNE AND ORLANDO J ANDY

Since the use of hypaque for cerebral angiography is relatively recent it was thought advisable to give a preliminary report on the number and types of reactions observed with this substance

METHOD

The results in this study were based on 222 arterial examinations in 128 patients The examination consisted of AP and lateral views (each necessitating separate injections) and oblique views in the cases of aneurysms The number of injections necessary to obtain these views with a single arterial puncture made up one examination The patients ages ranged from 27 to 71 years Routine preliminary medication consisted of 60 mg of phenobarbital All examinations were done by percutaneous needle insertion under local anesthesia (1% xylocaine) in the awake patient Five to 10 cc of 50% solution of hypaque (sodium 3 5–diacetamido–2 4 6–triiodobenzoate) were injected rapidly for each view

RESULTS

Complications occurred in 9 of the 128 patients (7%) These occurred during or immediately after the completion of the injections All complications were transient (5 hours was the longest duration) None occurred with the six vertebral arteriograms

The complications consisted of hemipareses 6 convulsions 2 visual disturbances 1 unconsciousness 1

Papaverine HCl (60 mg) was given intraarterially and intramuscularly (120 mg) to the cases of hemiplegia

DISCUSSION

One patient (age 37) developed transient focal seizures and hemiparesis contralateral to the injection His brief hemiparesis may have represented a postictal phenomena The other patient (age 43) who had a seizure had had a focal seizure prior to admission

Of the 6 patients who developed hemiparesis one (50 years old post traumatic) had a mild hemiparesis on admission which transiently became more marked during the examination Another (age 30) had a transient hemiparesis before admission This was on the same side as the hemiparesis which developed during injection

The visual disturbances mentioned consisted of streaks of yellow light which got more intense then stopped suddenly in a few seconds after the right internal carotid was injected When the left carotid artery was injected momentarily everything got white and the patient felt pain in the left ear This 36 year old patient had an aneurysm of the right internal carotid artery for which the neck was ligated Postoperative arteriography (right side) did not cause any reaction

* From the Division of Neurosurgery Department of Surgery The University of Mississippi Medical Center and Veterans Administration Center

The transient period of unconsciousness occurred in a 71 year old patient who suffered from a subarachnoid hemorrhage and aphasia. The patient voided during the brief time of the reaction.

The ages of the 9 patients with reactions were 27, 30, 36, 37, 43, 48, 50, 62 and 71 years. The predominance of the reactions in the age group under 50 is at variance with our previous experience with diodrast. However, the series is too small to offer anything but impressions.

That all the reactions were transient suggests that the reactions are due to a temporary vascular insufficiency, very likely secondary to spasm. It is of interest that only 1 of the 9 patients developed their reaction on the first injection of hypaque. Also because of their transient nature, we feel most of these reactions would not have been observed if the patient had been under general anesthesia during the procedure.

The incidence of reactions is only slightly less in our hands with hypaque (7%) than with 35% diodrast (9%) but there were no prolonged reactions as we have seen with diodrast.

SUMMARY

A series of 9 reactions in 128 patients undergoing 222 arterial examinations is presented and discussed.

THE DESIGN AND APPLICATION OF A HYDRAULIC ARTERIAL CLAMP FOR EXTERNAL CONTROL OF CEREBRAL BLOOD FLOW*

ROBERT J. WHITE, ALFRED UIHLEIN, AND JOHN H. GRINDLAY

Direct control of cerebral blood flow would facilitate greatly the surgical manipulation and obliteration of aneurysms and other vascular anomalies of the cerebral circulation. It would be of particular value during the use of hypothermia when the brain can tolerate significant reduction in oxygenation for short periods.[1, 2] Since the intracranial circulation in the human is furnished almost exclusively by the carotid and vertebral arteries, cerebral blood flow can be influenced directly by controlling circulation through these vessels in the neck. Recently we have developed an arterial clamp, known as the "hydraulic arterial clamp," which gives promise of reducing cerebral blood flow rapidly, effectively and safely when applied to these extracranial arteries.

Construction. The instrument is of simple design and manufacture and was constructed by the Section of Engineering, Mayo Clinic. The essential parts consist of a polyethylene holder shaped like a hollowed out wheel cut almost through diametrically and bearing one or two deep circumferential grooves on its external surface, and an inflatable latex open type collar molded in one piece to a 25 cm. tube ending in a "V" (Figs. 1 and 2). The

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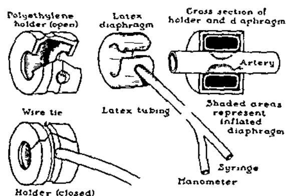


Fig 1 Schematic drawing of component parts of hydraulic arterial clamp polyethylene holder, latex collar, and connecting tube method of closure to occlude vessel



Fig 2 Photograph of polyethylene holder, latex collar, and Y tubing

tubing is reinforced with several extra coatings of rubber The collar fits snugly into the hollow of the polyethylene holder The holder and collar are slipped about an artery and the holder closed with steel wire tied securely in the external groove (or grooves) One of the "Y" endings is connected to a syringe containing sterile saline and a small quantity of *radiopaque material (hypaque sodium, 50%), the other end to a manometer* (or this end is simply closed) The desired degree of occlusion can be produced by increment or decrement of fluid pressure in the collar Its design permits the construction of the hydraulic arterial clamp to be varied to fit any vessel of significant size Between uses the clamp is cleaned in lukewarm water and dry stored Prior to use it is soaked in antibiotic solution for 8 hours

RESULTS AND COMMENT

To date studies using this clamp have been carried out successfully in 10 dogs and 4 humans After suitable surgical exposure of the vessels it was used to occlude partially or totally the carotid, femoral and iliac arteries in dogs and the common carotid artery in humans The efficacy of the clamp was reflected in its control of intracranial bleeding following rupture of a cerebral aneurysm in one human case No evidence of embolization or thrombosis has developed in either humans or dogs following the use of clamps for periods up to 6 hours (Fig 3) Wound infection has not occurred The design makes possible complete occlusion or total reduction of applied pressure in less than 1 second without reentry into the surgical wound Likewise the clamp itself can be removed with ease For best results the polyethylene holder should fit the vessel wall snugly and the entire hydraulic system should be checked before application to determine the desired amount of fluid required for closure (in our studies 1 to 2 cc)

Recently Jacobson and McAllister [3] have designed an excellent pneumatic cuff which has been used successfully in permanently occluding large blood vessels in dogs Although this cuff, so far as we know, has not been used to occlude the carotid circulation—probably because of its size and method of closure—it does offer the advantage of single piece construction



Fig 5 Photograph made at operation with the hydraulic arterial clamp in place about the common carotid artery in the human

The hydraulic arterial clamp seems applicable in cases requiring gradual occlusion of the carotid artery prior to subsequent ligation for cerebral aneurysm Here the graded reduction in size of arterial lumen could be measured manometrically and, most importantly, arterial compression could be released immediately if signs of cerebral ischemia developed

The presence of a radiopaque medium within the confines of the collar makes possible roentgenologic verification of the degree of vascular occlusion produced Since the arterial clamp has not been left for long periods within the tissues of the neck, it is impossible to be sure of its ultimate effect on the vessel wall and the surrounding tissues Because of its design, however, rupture of the vessel might be produced by torque and vascular scarring.

Presently we are preparing to control circulation through the carotid and vertebral systems simultaneously, thus stopping cerebral blood flow for short periods under hypothermic conditions during intracranial vascular operations

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THE CLINICAL SIGNIFICANCE OF VARIATIONS IN THE CERVICOTHORACIC AND MIDDLE CERVICAL GANGLIA OF THE SYMPATHETIC TRUNK AND OF PREGANGLIONIC SYMPATHETIC FIBERS OF THE CERVICAL NERVES OF MAN *

RAEBURN C LLEWELLYN EDWARD McC PEEBLES AND HOMER D KIRGIS

Factors contributing to unsatisfactory results following sympathectomy of the upper extremity include 1) incomplete interruption of sympathetic pathways 2) functional regeneration of preganglionic fibers 3) progression of the disease process such as an arteritis despite the absence of sympathetic innervation and 4) sensitization of sympathectomized smooth muscle to epinephrine or epinephrine like substances

Kuntz[1] and others[2 3 4] have described inconstant sympathetic pathways to the upper extremity that may not be interrupted by removal of the cervicothoracic ganglion alone or by removal of the cervicothoracic and second thoracic ganglia Conflicting descriptions[5 6 7 8 9 10] of the anatomy of this portion of the sympathetic nervous system have contributed to the performance of inadequate surgical procedures

The present work has been done to reevaluate the role of aberrant sympathetic pathways and functional regeneration of preganglionic fibers in the failure of sympathectomy to provide more complete and more lasting clinical benefits

METHOD

The cervical and upper thoracic portions of 62 pairs of sympathetic trunks were dissected at autopsy or in the anatomy laboratory In addition microscopic studies have been made of the intradural portions of the anterior roots of the seventh and eighth cervical and upper thoracic spinal nerves

Gross Dissections The most common gross ganglionic pattern of the upper thoracic and cervical portions of the sympathetic trunk consisted of a superior cervical a lower middle cervical a vertebral and a cervicothoracic ganglion (Fig 1) The second most common ganglionic pattern included a superior cervical an *upper middle cervical a lower middle cervical a vertebral and a* cervicothoracic ganglion Figure 2 illustrates the relations of the upper and middle cervical and vertebral ganglion in this pattern The third most common ganglionic pattern consisted of a superior cervical an upper middle cervical a vertebral and a cervicothoracic ganglion (Fig 3)

The middle cervical ganglion was classified as an *upper* type when located opposite or above the carotid tubercle It was classified as a *lower* type when it lay inferior to the carotid tubercle Its most common position was immediately inferior to the carotid tubercle opposite the sixth cervical intervertebral space In this position it lay in close relationship to the inferior *thyroid artery*

The vertebral ganglion was present in all dissections but was fused with the cervicothoracic ganglion in five trunks This ganglion was quite variable

* From the Departments of Surgery (Neurologic Surgery) and Anatomy Tulane University School of Medicine New Orleans Supported by a research grant (PHS B 1052(C2) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health U S Public Health Service



Fig 1

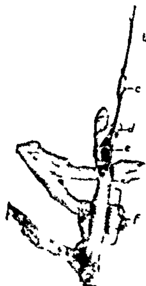


Fig 2

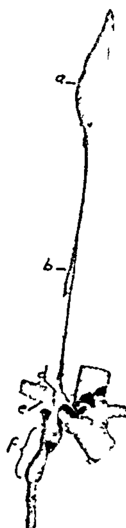


Fig 3

Gross ganglionic patterns of cervical and upper thoracic sympathetic trunk Key to legends *a* superior cervical ganglion *b* upper middle cervical ganglion *c* lower middle cervical ganglion *d* vertebral ganglion *e* vertebral artery *f* cervicothoracic ganglion

in size often being very small It always lay in close contact with the vertebral artery Also in all dissections it was connected with the cervicothoracic or inferior cervical ganglion by two small trunks one of which passed posterior to the vertebral artery and the other anterior to the artery The trunk which passed posterior to the artery was larger than the anterior trunk In those instances in which the vertebral ganglion was fused with the inferior cervical or cervicothoracic ganglion it lay posterior to the vertebral artery The anterior trunk in each such case connected the upper pole of the cervicothoracic ganglion with the upper pole of the vertebral ganglion

Partial segmentation of the cervicothoracic ganglion into an inferior cervical and a first thoracic ganglion was noted in many instances These ganglia were distinctly separated in only 11 cases

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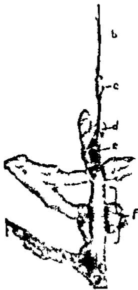


Fig 2

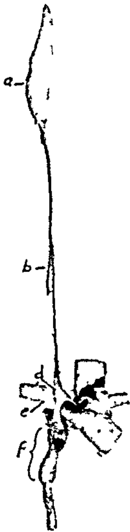


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Microscopic Preparations. Microscopic preparations consisting of transverse sections of the intradural portions of the cervical and upper thoracic spinal nerves were analyzed for the presence of preganglionic fibers. Preliminary studies revealed that preganglionic fibers may be present in the seventh and eighth cervical nerves (Fig 4) but the number in each such nerve studied was small. The number of preganglionic fibers in the first thoracic nerve of man appears to vary widely

DISCUSSION AND CONCLUSIONS

Although it has been stated that the upper extremity can be satisfactorily isolated from central sympathetic centers even though the cervicothoracic ganglion and its rami are allowed to remain intact, the currently conventional sympathectomy for the upper extremity consists of removal of this ganglion plus the second and sometimes the third thoracic sympathetic ganglion This type of sympathectomy gives satisfactory results quite consistently. However, there is a sufficiently high incidence of either incomplete isolation of the upper extremity from central sympathetic centers or in the recovery of sympathetic function in the extremity following an apparently complete sympathectomy to warrant the conclusion that there are sympathetic pathways quite commonly present that are not interrupted by this type of sympathectomy or there are ganglia into which functional regeneration of the severed preganglionic fibers can occur easily. It seems probable that the latter reaction is the cause of the majority of unfavorable results following sympathectomy other than those due to progression of pathology in the extremity

The present investigation has demonstrated that there may be preganglionic

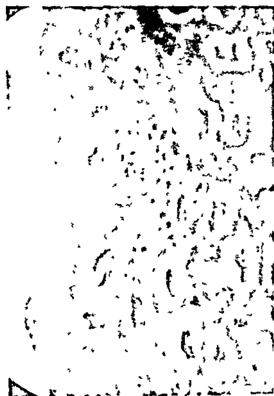


Fig 4 Cross section of intradural portion eighth cervical nerve showing preganglionic fibers

fibers in both the seventh and eighth cervical spinal nerves. Most of the communicating rami that extend between these nerves and the sympathetic trunk or ganglia, which from their gross position would suggest they might contain preganglionic fibers, are related to the cervicothoracic ganglion. Some rami of this category pass directly to the vertebral or middle cervical ganglion. Therefore, if the vertebral and lower middle cervical ganglia are not removed at operation, all pathways to the upper extremity may not be interrupted. In any event, if not removed, these ganglia offer an available point at which regenerating preganglionic fibers may establish functional connections.

We believed that regeneration of preganglionic fibers, severed by removal of the cervicothoracic and second thoracic ganglia, into the vertebral and lower middle cervical ganglia is the major factor in the recovery of sympathetic activity in the upper extremity following the sympathectomy conventionally performed. It is suggested that sympathectomy for the upper extremity include removal of the vertebral ganglia and, if present, the lower middle cervical ganglion as well as the cervicothoracic, the second and the third thoracic ganglia.

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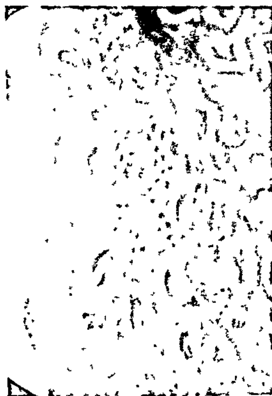


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Orthopedic Surgery

THE MOTOR DENERVATED LIMB OF THE RAT AS A TOOL IN THE STUDY OF THE REPARATIVE PROCESS IN OSTEOPOROTIC BONE *

WILLIAM S SMITH, JOHN L WOLFF, CARL R COLEMAN AND RICHARD E SLAGER

The object of this report is to further explore the use of the denervated limb as a tool in the reparative process of osteoporotic bone

In a report before the Surgical Forum [1] in 1955 roentgenographic evidence was presented to suggest delayed union of fractures of rat tibiae previously rendered atrophic by sciatic denervation Armstrong [2] showed that the ash content of denervated rat limbs could be maintained if estradiol dipropionate was administered concomitantly Based upon these two observations, the present study was formulated to observe certain aspects of the effect of sex hormones upon fracture healing in a limb made osteoporotic by motor denervation

Three phases of this problem were studied 1) comparison of the p^{32} uptake of the control and fractured limbs as one index of local metabolic activity following hormonal administration, 2) comparison of radiographs at various stages of the reparative process, 3) the effect of sex hormones upon the weight of the denervated, fractured limbs

METHOD

Delatestryl and delestrogen were the hormones selected for the study Delatestryl or testosterone 17 beta n nenanthate is a long acting testosterone Delestrogen or estradiol 17 beta n valerate is a long acting estrogen Previous studies in rats have indicated that a significant endocrine effect could be expected for at least five weeks from both drugs in the doses that were selected [3] To insure a prolonged and continuous action of the hormones a second identical dose was given at 27 days after the first injection

Twenty four hours before sacrificing the animals, each rat received an intra peritoneal injection of p^{32} in a dose calculated at 0 25 μc/gm This, therefore, was equal to 10 μc in the case of a 400 gm rat Since we were comparing the ratio of the radioactive phosphorus activity per gram of bone in the frac tured to the nonfractured limb of the same rat small variations in dosage were not significant This dose is less than that used by some workers, but it gave workable counts with our equipment and was low enough to obviate the problem of disposing of highly radioactive rats after sacrifice

Ninety nine Sprague Dawley white rats, 8 weeks in age, were kept under similar conditions of temperature, diet, and quarters Right sciatic denerva tion was performed in 63 of the rats The right tibia and fibula of all 99 rats

* From the Department of Surgery, Division of Orthopedic Surgery The Ohio State University College of Medicine Supported by a grant from the Ohio State University Development Fund

were manually fractured 35 days later. They were then divided into 5 groups: (*A*) Fifteen animals which were given no medications and did not have sciatic denervation. (*B*) Twenty one animals which received 0.08 mg of delestrogen and did not have sciatic denervation. (*C*) Twenty one animals which received no medications but had sciatic denervation. (*D*) Twenty one animals which received 0.08 mg of delestrogen and had sciatic denervation. (*E*) Twenty one animals which received 20 mg of delatestryl and had sciatic denervation.

Animals in each group were sacrificed at 25, 40 and 55 days following their fractures. After sacrificing the animals, the fractured and control tibia and fibula were disarticulated at the knee and ankle joints. The soft tissue was immediately dissected off the bones and discarded and the bones were accurately weighed. The specimen was then placed in a plastic counting chamber in which its entire length was covered by the head of the Geiger counter. To obtain accurate counts the specimen was rotated 180° about both its longitudinal and transverse axis and 4 readings were recorded. With the use of this method of 4 positions the highest count obtained was never more than 10% greater than the lowest count. The ratio of the counts per gram of bone in the fractured as compared to the control side was used in recording the activity (Fig. 1).

RESULTS

Groups *A* and *B*. The animals with intact sciatic nerves were compared. Group *A* rats had received no medication and Group *B* had been given delestrogen. At 25 days (Fig. 2) the limbs of estrogen treated animals had a significantly higher p^{32} uptake ratio than those which received no medication.

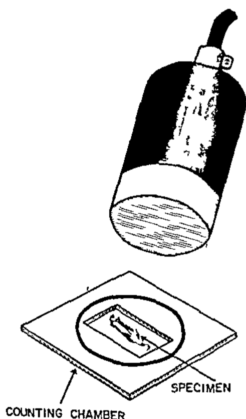


Fig. 1

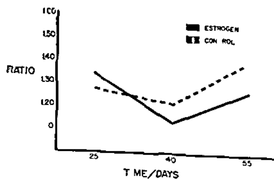


Fig. 2

At 40 days the ratio had become lowest in the estrogen treated animals The downward trend, as compared to the animals which had received no medication continued in the 55 day rats However at 40 days and at 55 days the P^{32} uptake in the estrogen treated limb is closer to that of the control side This suggests that in the estrogen treated animals fracture healing has progressed to a point which more closely approximated the nonfractured extremity

There was no appreciable difference in the weights of the limbs in the estrogen or control groups

Groups *C* and *D*. Groups *C* and *D* rats had their sciatic nerves sectioned The Group *D* animals were also given delestrogen At 25 days (Fig 3) there was only a minor elevation of the p^{32} uptake ratio in the estrogen treated animals as compared to the controls of Group *C* The activity was equal in the two groups at 40 days However, at 55 days the ratios were 1 37 for the control and 1 07 for the estrogen treated animals Since the ratio in the latter group approaches 1, it is apparent that the metabolic activity of the fractured limb is very near that of the control side at 55 days

Weights of the limbs in the two groups of animals did not vary significantly

Radiographs of the 55 day postfracture estrogen treated denervated animals showed increased density of the callus, less distinct fracture lines and more advanced remodeling than all other groups (Fig 4)

Groups *C* and *E*. Group *C* and *E* rats had sciatic denervations The Group *E* rats however, had delatestryl Figure 5 shows that the highest ratios of the study were in the 25 day rats treated with testosterone It was noted that the ratio in the testosterone animals at 40 and 55 days remained above the ratio in the control animals However, the difference was not significant The ratios at 55 days did not approach 1

The weights of the testosterone treated tibiae and fibulae were 11% heavier than those of the control animals at 40 days There was no difference at 25 days and 55 days No explanation can be given for this difference except that

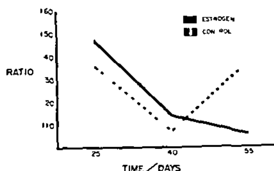


Fig 3

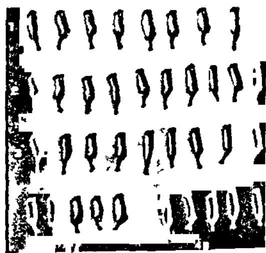
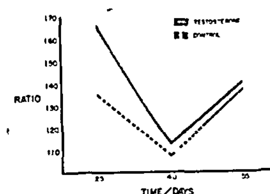


Fig 4 X ray of tibiae 55 days after fracture Sciatic nerve resection performed in animals in Groups 1 2 and 3 35 days prior to fracture (No denervation in Groups 4A and 4B) Group 2 treated with androgen Groups 3 and 4A treated with estrogen For detailed view see Figure 5

Fig 5



testosterone may initially increase the metabolic activity in the fractured limb that has been denervated

DISCUSSION

The range of error in this preliminary report of the radioactivity recordings fell within the range of error of the counters and recorders, 1 to 5%

There were two disturbing findings in this study First, there was a uniform depression of all ratios at 10 days This occurred in normal as well as denervated limbs and in the control as well as medicated animals Secondly, the tibia and fibula of all the 10 day rats, including the controls, weighed even less than the 25 and 55 day rats Whether this is a laboratory discrepancy such as a temporary dietary deficiency or part of a normal pattern remains to be proven

SUMMARY

Evidence has been presented to suggest the feasibility of using the motor denervated limb of the rat in the study of the reparative process in osteoporotic bone By the methods demonstrated, the fractured tibiae of denervated estrogen treated rats appeared to more nearly simulate the control limb than did testosterone or untreated rats at 55 days The authors believe that further investigation of this problem is warranted

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A STUDY OF THE GROWTH AND NUTRITION OF SURGICALLY CREATED OSTEOCHONDRAL LOOSE BODIES IN ADULT AND YOUNG RABBITS *

ROBERT W BAILEY AND ROBERT E SELLE

The mechanism of survival and further growth of clinically occurring intra articular loose bodies composed of cartilage or bone plus cartilage served as a stimulus for this study Loose bodies may be encountered as a result of osteochondral fractures osteochondritis dissecans, etc The authors have ob served a disparity in size between a loose body and the defect from which it arose in instances of osteochondritis dissecans which suggested growth of the loose body after its detachment The mechanism by which this could occur and the validity of this observation seemed worthy of further study

Experimental work has been previously conducted on the fate of surgically created osteochondral loose bodies by Axhausen [1] Coronil and Coudray,[6] Rimann [12] and Fisher [9] None was more than a short term experiment, largely histologic Fisher believed that the majority of bone cells died while the hyaline cartilage cells had survived 5 weeks following removal of a section of the femoral condyle and its placement into the suprapatellar pouch

METHOD

Eighteen rabbits 9 of them ranging in age from 4 to 8 weeks and 9 young adult animals, were used They were divided into 3 groups of 6 each with 3 adult and 3 young in each group These were labeled Groups 1 2 and 3

All 18 were treated in the following manner An arthrotomy was performed on the left knee of each A special bone cover was developed by means of which a fairly constant section of articular cartilage ¼ in in diameter and ⅛ in thick was removed from the medial femoral condyle Under sterile conditions the section was wet weighed and placed in the intercondylar area following which the knee joint was closed During the first postoperative week roent genograms were made of the knee in anteroposterior and lateral projections These were repeated a short time before sacrifice of the animals to note any change in position of the section None was observed

Six weeks following surgery, the animals in Group 1 were treated by the injection of 300 microcuries of $H_2S^{35}O_4$ (as the sodium salt) into a large ear vein [2, 3, 5, 7, 9] Forty eight hours later, the animals were sacrificed and the joints were studied in respect to changes in the area of the femoral condyle from which the loose body had been removed The location of the loose body its gross appearance and wet weight were noted

One half of the loose bodies were used in making histological sections and autoradiographs On the remaining half measurement of radiosulfur was per formed by employing the nitric acid wet *digestion technique designed to* oxidize all organic sulfur occurring as chondroitin sulfate to an inorganic form prior to its precipitation as barium sulfate This method was a modifi cation of the Jowsey [10] technique Counting was carried out using a thin mica

* From the University of California Medical Center Los Angeles with assistance from the United States Public Health Service Grant A 2406 With the technical assistance of B Wellington Cleaver

window counter The results were expressed in counts per minute (cpm) per milligrams of tissue

Groups 2 and 3 were studied in a similar manner the former at 2 months the latter, 6 months postoperatively One animal in the adult group died within the first postoperative week The remaining 17 survived until the time of sacrifice

RESULTS

In the 17 joints examined at the completion of the study the loose body was identified and removed in 16 In 1 animal no remnant of the body could be found This was a young rabbit sacrificed 6 months postoperatively In only 2 rabbits was any soft tissue attachment to the loose body observed In the remainder the loose body was recovered from where it had been initially placed In the 2 instances where a pedicular attachment was present a fine synovial filament loosely stretched from the retropatellar fat and extended to the loose body

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surface was shiny and glistening as though covered by fibrocartilage

The wet weight of the specimens at the time of sacrifice was compared with that at the time of operation The differences are tabulated in Table 1 All

Table 1 Gain in Weight of Loose Body

	GRAMS	WEEKS POST-OP
GROUP 1		
ADULT	0 001	4 WEEKS
YOUNG	0 001	
GROUP 2		
ADULT	0 004	8 WEEKS
YOUNG	0 0046	
GROUP 3		
ADULT	0 0065	24 WEEKS
YOUNG †	0 013	

† In 1 rabbit loose body could not be found

specimens showed an increase in weight that was distinctly greater the longer the duration of time following surgery The net gain was 1 mg at 4 weeks postoperative and 4 mg 8 weeks postoperative in both adult and young groups In Group 3 the net gain was 6 5 mg in the adult and 13 0 mg in the young group

The special corer had resulted in a rather uniform specimen whose average weight was 36 0 mg Expressed as percentage weight gain the adult Groups 1 2 and 3 showed an average gain of 3 5 mg or 9 7% the young Groups 1 2 and 3 6 2 mg or 17 0% These changes seemed of statistical significance (Table 2)

Histologic sections were made from those specimens set aside for autoradiographs An error in technique resulted in the leeching of S^{35} from the specimens and as a result the autoradiographs were not of acceptable qualitative

Table 2 Average Weight Gain of Loose Bodies

	GRAMS	PER CENT
ADULT GROUPS		
1 2 and 3	0 0035	9 7%
YOUNG GROUPS		
1 2 and 3	0 0062	17%

value At 2 months (Fig 1) on the bone side of the specimen, a fine fibrous tissue covering was present At 6 months (Fig 2, 3) this was well developed and *presented in the gross as a shiny fibrocartilage like covering* On micro scopic section, the fibrous tissue was well developed with occasional evidence of intramembranous new bone formation observed especially at the peripheral area of junction of bone and cartilage [11] Although, in the gross the fibrous covering resembled fibrocartilage, even at 6 months the histologic picture was not clearly that of true fibrocartilage



Fig 1 Photomicrograph entire loose body at 6 weeks Hyaline cartilage appears viable A fine layer of fibrous tissue covers bone side



Fig 2 Photomicrograph 8 weeks Note fairly well developed fibrous tissue over bone probably arising from marrow elements Necrosis of peripheral bone



Fig 3 Photomicrograph 6 months Very well developed fibrous tissue over bone surface appearing fibrocartilaginous in some areas *Intramembranous* new bone formation present near junction of bone and cartilage

The gain in weight of the specimen when correlated with the histologic picture seemed to be explainable on the basis of the fibrous tissue proliferation In none of the specimens either in the young or adult group was there evidence of growth of hyaline cartilage but distinct evidence of its survival

The defect from which the loose body had been removed from the femoral condyle became covered by fibrous tissue which at 6 months histologically appeared to be fibrocartilage

Measurement of radiosulfur in the specimens was performed by use of the nitric acid wet digestion technique designed to oxidize all organic sulfur present to an inorganic form followed by its precipitation as barium sulfate Tabulations were made using the flow counting technique expressed as counts/min /mg of tissue These figures were converted to microcuries/gm of sample The accompanying table indicates the result of this phase of the study (Table 3)

Table 3 Measurement S^{35} in Loose Body

	MICROCURIES/GM SAMPLE
ADULT RABBITS	
GROUP 1	8.8 x 10-
GROUP 2	NO SAMPLES
GROUP 3	10.9×10^2
YOUNG RABBITS	
GROUP 1	2.8×10^2
GROUP 2	3 1 x 10
GROUP 3	2 $\times 10^2$

The quantity of S^{3} recovered in any of the specimens was small but it would seem that one fact stood out—the amount of S^{35} recovered in the loose body in the adult animal was considerably greater than in the group of young rabbits Secondly the duration of time between surgery and sacrifice of the animals had little effect on the amount of sulfur recovered from the loose body

The reason for greater concentration of S^{35} in the adult group over the young group is not clear Comparative values of the concentration of S^{35} in other areas such as the articular cartilage of another joint as the epiphyseal plate were not made in this experiment It is possible that in the young the demands for sulfur in the synthesis of chondroitin sulfate are greater in other areas e g the epiphyseal cartilage and as a result there is less available to the loose body

CONCLUSIONS

1 The loose body appeared to increase in size with no relation to the age of the animal largely on the basis of fibrous tissue proliferation along its bony side The degree of fibrous tissue proliferation was more marked the greater the — '
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At . . . time this was associated with some new intramembranous bone formation especially at the peripheral junction of bone and cartilage

3 The hyaline cartilage appeared viable histologically in all specimens but no evidence of cartilaginous growth was observed

4 The loose body exhibited a significant amount of S^{35} uptake greater in the older than the younger animal The significance of this latter observation is not clear The means by which the S^{35} reached the loose body appeared via the synovial fluid since no significant synovial pedicle was observed which might suggest origin of the S^{35} from blood serum

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FAT EMBOLISM CHANGES IN THE SERUM LIPASE LEVELS OF PATIENTS AFTER FRESH FRACTURES AND ORTHOPEDIC OPERATIONS *

LEONARD F PELTIER AND SING-PING LAI

The need for a laboratory method for establishing the diagnosis of fat embolism is emphasized by the fact that although fat embolism is the most frequent cause of death following fractures this diagnosis is rarely made in the living patient

In 1940 Struppler[10] noted an elevation of the serum lipase and tributyrinase in patients following fractures Two of his patients with elevations of the serum lipase were found to have fat embolism at autopsy Since that time there have been other studies of the serum tributyrinase levels in patients following fractures[3 4 8 9 11] the most important of which is that of Schuttemeyer[7] We have studied the serum tributyrinase levels in dogs following the intravenous injection of various substances and found that such elevations occur only when diffuse pulmonary embolism with neutral fat has occurred[6]

Serum tributyrinase levels increase within a few hours after fracture and return to normal within 24 to 48 hours Because the embolic fat from the bone

* From James B Weaver Laboratory for Orthopedic Surgery University of Kansas Medical Center Kansas City Kans Supported by Grant #H 3592 from the U S P H S

is neutral fat[6] and not tributyrin and since changes in the serum tributyrinase levels do not closely parallel the clinical course of patients with fat embolism we decided to investigate further the serum lipase levels in patients following fractures and elective orthopedic operations

METHOD

The serum lipase levels were determined by the method of Cherry and Crandall[1] as modified by Comfort and Osterberg[2] The method consists essentially of the incubation of 1.0 ml of serum with a buffered olive oil emulsion at 37°C for 24 hours The free fatty acids released by hydrolysis are then titrated to an end point with 0.5N NaOH The results are reported in terms of milliliters of 0.5N NaOH In our laboratory 81 normal samples were examined as controls 95% of these fell into a range of 0.0 to 0.85 ml of 0.5N NaOH In this study we have considered any serum lipase level in excess of 1.0 ml to be significantly elevated

Blood was drawn using disposable plastic syringes transferred to centrifuge tubes allowed to clot and then centrifuged If the determination was not begun immediately the serum was frozen Blood samples from emergency cases were drawn immediately on admission Blood samples from elective surgical cases were drawn in the operating room at the time anesthesia was begun Subsequent samples were drawn early in the morning usually before breakfast had been served Blood samples were obtained on the day of admission or operation and on at least 4 successive days When the serum lipase was significantly elevated additional samples were drawn

RESULTS

Seventy five patients are included in this report—50 men 25 women Twenty of these underwent elective orthopedic operations 48 suffered fractures of only 1 bone 7 had multiple fractures None of these patients suffered abdominal trauma with possible damage to the pancreas The administration of anesthetic agents did not appear to affect the results In 16 of these 75 patients the serum lipase level became significantly elevated In only 1 a house painter with a fracture of the os calcis was the serum lipase found to be elevated above 1.0 ml on the initial examination (See Table 1) No patient with a minor injury to bone or with injuries limited to soft tissue subsequently had a significant elevation of the serum lipase

In most cases the serum lipase did not reach its maximum level until at least 72 hours after fracture or operation and in those patients with marked elevations of the serum lipase the level remained above normal for as long as 2 weeks or more This is in marked contrast to the reported behavior of the serum tributyrinase levels

In some patients the serum lipase levels never exceeded 1.0 ml although starting with a low initial value the successive samples showed a definite increase in the serum lipase levels Multiple samples indicating a pronounced upward trend from a low initial value appear to have as much significance as isolated samples with values above 1.0 ml

Three patients with extracapsular fractures of the hip came to autopsy In 1 whose serum lipase was never elevated no fat emboli were found In the other 2 whose serum lipase became significantly elevated fat emboli were found in both

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Table 1 The Incidence of Significant Elevations of the Serum Lipase in Patients with Fractures and Orthopedic Operations

DIAGNOSIS	NO OF PATIENTS	NO WITH ELEVATION
Multiple fractures		
tibiae hip (extracapsular)	1	
acetabulum clavicle	1	
dislocation hip acetabulum	2	1
femur tibia & fibula L 4	1	1
femur tibia & fibula os calcis	1	1
ribs (8)	1	1
	7	4
Single fractures		
rib	1	
spine	3	
pelvis	1	
Colles	1	
hip intracacapsular	7	
extracapsular	15	7
femur shaft	7	2
patella	1	
tibia & fibula	5	
ankle	3	1
os calcis	4	1
	48	11
Elective orthopedic operations		
osteotomy femur	6	1
insertion prosthesis hip	1	
insertion pins femoral neck	1	
amputation femur	1	
arthrotomy knee	3	
sequestrectomy	2	
subastragular fusion	1	
debridement ankle	2	
wrist fusion bone graft	1	
non union fracture forearm bone graft	1	
excision of soft tissue sarcoma	1	
	20	1
Total	75	16

DISCUSSION

On the basis of animal experiments [illegible] there is no doubt that the increase in serum tributyrinase is the result of hypersecretion by the parenchymal cells of the lung in response to the presence of large amounts of embolic neutral fat The increase in the serum lipase noted in these patients can be explained on a similar basis There is a very good correlation between the degree of bone trauma and the subsequent elevation of the serum lipase

It is not possible to predict which patients will have clinically evident fat embolism by the determination of the serum lipase One can, however, diagnose fat embolism in the confused, semiconscious patient with fractures and possible head injuries, if the serum lipase is elevated above 1 0 ml and there are no localizing neurological signs

CONCLUSIONS

In patients with trauma to bone either from fractures or orthopedic operations, there is a subsequent elevation of the serum lipase level which appears to be proportional to the amount or degree of bone trauma Under some circumstances an elevation of the serum lipase may be used to establish a presumptive diagnosis of fat embolism

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THERAPY OF TRAUMATIC FAT EMBOLISM WITH INTRAVENOUS FLUIDS AND HEPARIN *

CULLY A COBB, JR, VIRGIL S LEQUIRE, MARY E GRAY AND J WILLIAM HILLMAN

Embolization of fat is a frequently fatal complication of trauma producing fractures or massive soft tissue injury [1,2] Clinical manifestations are tachycardia, tachypnea pyrexia, delirium, stupor and decerebrate rigidity.[3] In this study emboli tagged with I^{131} were introduced into veins and arteries of dogs to determine 1) the pattern of distribution of the fat, 2) the rate of metabolism of the emboli, 3) factors leading to accelerated mobilization and metabolism The information secured has been applied in a series of patients having clinical fat embolism following long bone fractures

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* From the S R Light Laboratory for Surgical Research, Divisions of Neurosurgery and Orthopedic Surgery Department of Surgery and Department of Anatomy, Vanderbilt University School of Medicine Nashville Supported by Public Health Grant #H 1570 and funds from the John B Howe Fund for Research in Neurosurgery

METHOD

Neutral fat, either dog fat or olive oil, was iodinated with I^{131} by the method of Stanley and Thannhauser[4] Preliminary experiments showed that widely varying concentrations of embolic fat could be located by surface counting of gamma activity It was apparent that variations in thickness or vascularity of the areas counted caused some distortion of the radioactivity over various structures Points were chosen for counting in subsequent experiments and measurements of radioactivity were made after injection of sodium iodide in 6 dogs Figure 1 shows the positions counted With activity over the lung as unity correcting factors for other areas are liver 1 1, hindquarter 1 47, thyroid 1 67, brain 1 50 These measurements were made during the 4 hours following the injection and before significant concentration of the iodide in the thyroid gland Counts of radioactivity in embolism experiments were corrected by these factors

Radioactivity was measured with a Geiger Muller tube or with a collimated scintillation detector placed in contact with the skin Counts were corrected for background, decay, and body area, and expressed in percentages of initial activity over the lung

Distribution The purpose of this experiment was to determine the site and duration of arrest of embolic fat

In 9 dogs emboli of from 1 to 5 cc were injected into peripheral leg veins (Fig 2) All injections were made slowly over a period of about one minute An immediate very high level of radioactivity over the lung indicated almost complete arrest of the embolic fat

Gradual mobilization occurred in about 6 days with iodide absorption by the thyroid indicating metabolism of the fat The absence of a rise in radio activity over the thyroid in the first day showed that no significant amount of free iodide was available The point where the curves for lung activity and thyroid activity crossed thus provided a measurement of mobilization and metabolism of the embolic fat This point was reached in about 4 days

The possibility that other capillary beds might show characteristics differ ent from those in the lung was studied by injection of emboli into peripheral arteries Two femoral artery injections of 2 7 cc and 10 cc showed slight retention of emboli in the extremities but concentration in the lungs Five injections into the common carotid artery were then made (Fig 3 and Fig 4) It is seen that small quantities of embolic fat passed through the systemic

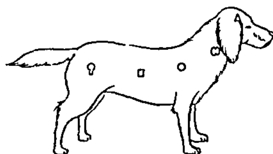


Fig 1 Location of points where radioactivity was measured

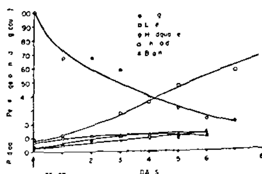


Fig 2 Venous fat embolism in 9 dogs

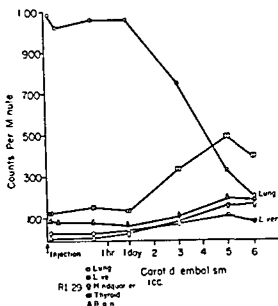


Fig.3 Embolism injected into carotid artery

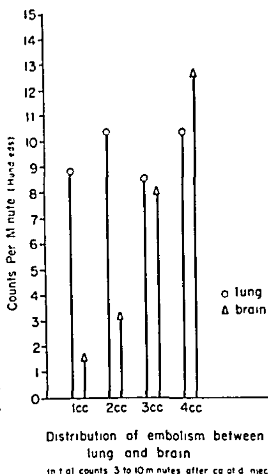


Fig 4 Proportions of carotid embolisms of increasing size in lung and brain

circulation and were arrested in the lungs With increasingly large emboli larger proportions of the fat were held in the head This study shows that while large amounts of fat may be arrested by the lungs, this filtration is not limited to the first passage of blood to the lungs but may arrest some emboli which have passed through the systemic circulation This suggests that the predominance of cerebral symptoms in fat embolism is not caused by a high degree of embolization of the brain but by an exceptional sensitivity to the effects of capillary embolism

Metabolism of Emboli Biliary fistulae were prepared in 4 dogs and bile and urine were collected during a period of observation following venous fat embolization In these animals sharply rising amounts of radioactivity appeared in the bile in about 30 minutes and persisted for up to 4 days The radioactivity of the bile reached levels as high as 20 times those for circulating blood collected at the same time A slower rise of radioactivity in the urine reached similar levels In these animals radioactivity over the thyroid was greatly reduced or absent (Fig 5) This indicated that drainage of the bile had reduced the availability of iodide One explanation of this was that fats or fatty acids were excreted in the bile and redigested to release iodide The other possibility was that metabolism of the fatty acids occurred in the liver with release of iodide in the bile Ether extraction of the bile showed radioactivity in both phases rising to higher levels in the aqueous phase after the

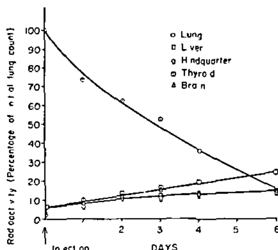


Fig 5 Fat embolism in 4 dogs with biliary fistulae

first twenty minutes These findings indicate that metabolism of the fatty acids may occur in both ways, partially by oxidation in the liver releasing iodide in the bile, and also by excretion of the lipids in the bile In either case the reduction of iodide available to the thyroid indicates that little hydrolysis of the neutral fat occurred before it reached the liver

Mobilization and Therapy. Emulsifying agents used for stabilizing nutrient fat emulsions were given intravenously and were found to have no effect on the pattern of venous embolism Agents used were Span 20, (sorbitan mono laurate) (Atlas Powder Co , Wilmington Del) and Demol 14, (a polyglycerol ester of oleic acid) (Emulsol Corp , Chicago) Heparinization led to a more rapid fall of radioactivity over the lungs and an earlier rise of thyroid activity (Fig 6 and Fig 7) The point of crossing of curves for lung and thyroid activity was reached in about 2 days instead of 4 It is probable that the lipemia clearing effect of heparin is responsible for this

Incorporation of these findings into our management of patients with long bone fractures led to the following program In patients suspected of having minor degrees of fat embolism, mainly those with multiple fractures of the major leg bones, manipulations or operations are delayed by approximately 5 days or more In patients not in shock who develop the syndrome of fat embolism oxygen therapy is instituted and intracranial hemorrhage is excluded by clinical findings, lumbar puncture, or exploratory burr holes [3] Where

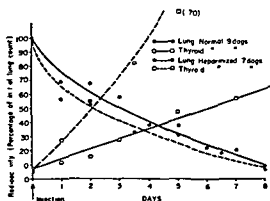


Fig 6 Comparison between normal and heparinized dogs

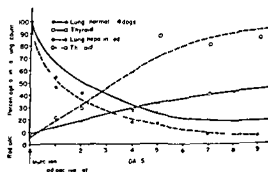


Fig 7 Simultaneous experiments with 1 cc emboli in normal and heparinized dogs

cardiac function is unimpaired parenteral fluid therapy up to 3 liters daily is then given.[5] If the signs of cerebral involvement progress and there is no evident risk of local hemorrhage, heparin is given intravenously in doses sufficient to raise the clotting time to between 20 and 40 minutes. Small doses given every 3 hours have been used in most cases. In a series of 19 cases 2 died acutely. Seventeen have recovered. Ten of these with progressive symptoms of cerebral involvement received heparin. Improvement was usually noted within 8 to 21 hours. Among these there has been no instance of hemorrhage. Studies of EEGs, psychological tests and work and school performance indicate that in nearly all cases survival of the fat embolism syndrome is followed by full recovery.

DISCUSSION

The lungs serve as a protective filter for the brain preventing embolic fat from reaching the central nervous system, and trapping some fat which has made the systemic circuit. Symptoms are probably caused by retardation of flow and local anoxia rather than particulate embolism. The embolic fat appears to be metabolized in the liver and partly excreted in the bile before metabolism of the fatty acids occurs. The administration of heparin leads to acceleration of the metabolism.

This study suggests that the period of latency before the onset of the fat embolism syndrome is the period required to overtax the capacity for filtration of the lungs. While a small amount of fat may enter the circulation in most fractures and many other injuries and illnesses usually no symptoms result. Except for the rare cases of clinical pulmonary embolism, the development of neurological changes follows the breakdown of effective pulmonary filtration. There is no direct evidence that toxicity of fatty acids, as mentioned by Newman[7] and Peltier,[8] contributes to the syndrome.

In normal fat metabolism the hydrolysis of neutral fats is carried out primarily by intracellular enzymes. The rapid formation of unesterified fatty acid from neutral fat is seen in intravenous infusions of nutrient emulsions in patients. Shoulders, Meng and Tuggle[6] have shown a sharp rise in blood fatty acids immediately following these infusions. A slight elevation of body temperature follows these infusions. In heparinized individuals fatty acid levels are increased approximately 3 times but the pyrexic response is reduced to approximately 1°F. The quantities of fat given in these infusions are greater than those in clinical or experimental fat embolism and because of the fine subdivision of the fat, metabolic processes are much more rapid. It is not apparent that any toxic effect can be assigned to the fatty acids produced.

The treatment of shock in patients with fat embolism has been stressed in the past.[7] The maintenance of circulating blood volume and urinary output, maximum oxygenation and, in our opinion, the acceleration of metabolism of emboli by heparinization give further protection to the central nervous system.

SUMMARY

Emboli tagged with I^{131} were introduced into veins and arteries of dogs to determine 1) the pattern of distribution of the fat, 2) the rate of metabolism of the emboli, 3) factors leading to accelerated mobilization and metabolism. The information secured was applied in a series of patients having fat embolism following long bone fractures.

Distribution. Almost all of the embolic fat introduced into a peripheral

vein is arrested in the lungs After about 4 days the emboli are mobilized and metabolism is indicated by the appearance of iodide activity in the thyroid Small emboli (1 to 2 cc) introduced into peripheral arteries or the carotid artery are not held in the peripheral vessels but rather in the lungs Increasing size leads to partial local arrest

Metabolism of emboli Examination of bile and urine following emboliza tion demonstrated substantial amounts of the tagged fat In animals with biliary fistulae the subsequent concentration of iodide in the thyroid was greatly reduced This suggests that metabolism of the fat occurs in the liver and that in part it is excreted in the bile and reabsorbed

Factors leading to rapid mobilization Emulsifying agents were tested with negative results Heparin led to a more rapid mobilization of embolic fat and an earlier appearance of iodide The use of heparin has appeared to imple ment recovery in patients with traumatic fat embolism Recovery has been estimated by physical and neurological signs, EEG changes, psychometric ex amination, school grades and work performance

We are indebted to Dr H C Meng for assistance in procedures and for other advice

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EXPERIMENTAL STUDIES ON THE STABILITY OF INTERNAL FIXATION OF FEMORAL NECK FRACTURES IN AUTOPSY BONE *

J PAUL HARVEY, JR, CARL HIRSCH AND PHILIP D WILSON

Internal fixation of femoral neck fractures was introduced by Nicolaysen in 1897, but it was not until Smith Petersen in 1931 and Sven Johanssen in 1932 that the method was accepted Although the use of internal fixation in fem oral neck fractures has improved the overall results there is still an appreciable number of failures

One well recognized factor which may be partially or even wholly respon sible for the poor results in femoral neck fractures is the anatomical route of the blood supply to the femoral head A subcapital fracture often results in partial or complete disruption of the vessels to the proximal fragment

* From the Biomechanical Laboratory Orthopaedic Service Royal Academic Hospital and Medical School Upsala Sweden

Although the vascular supply is of great importance we do not believe it is the only factor causing the poor results, for fixation and maintenance of close contact of fragments is also necessary to healing. Even in cases where avascular necrosis occurs, if the fragments are held firmly and in contact, healing of the fracture site and revascularization from the distal fragment will take place. Therefore, we feel that this mechanical aspect of the problem also deserves study.

Many different mechanical appliances have been devised to provide firm fixation and persistent contact. Single strong nails, multiple thin nails or pins, nails with plate attachment, screws, and recently screws with springs have been proposed and tested by application in clinical material. We feel that the mechanical features of the problem can be better studied in autopsy specimens. The sites of application and the best mechanical device can be carefully worked out prior to clinical usage.

Our initial efforts have been to devise a test which would approximate clinical conditions, yet be able to be studied by mechanical measurements and provide a means of comparing similar factors.

METHOD

The test as finally evolved, consisted of loading the intact specimen of an upper third of a human femur with a known force and measuring the deflection that occurred. The specimen then was sawed through the neck, nailed, and a force reapplied and the deflection recorded. We compared the values obtained in the intact specimen with the values obtained in the same specimen after nailing.

Bone from different specimens varied markedly in strength. Age of patient, degree of osteoporosis, architecture of bone, all are factors which cannot be controlled and which cannot be readily compared, specimen to specimen. However, if we merely mount a specimen in a compression machine, apply a force and observe the deflection occurring, then cut the same bone, apply an internal fixation device, mount the specimen in the same fashion, reapply the force and observe the deflection again, we can compare this deflection value in the same specimen under two different sets of circumstances without taking into account any extraneous factors as age, total strength, degree of osteoporosis, etc. Certainly these values can be grouped together and the results obtained used to draw conclusions.

We sawed the specimens at 90° to the axis of the neck just distal to the head. Although Bachman in his monograph [1] has shown that fractures similar to clinical types can be obtained in autopsy specimens by use of a compression machine, the occurrence of clinical types of fractures cannot be obtained consistently. Also to eliminate as many extraneous and unmeasurable factors as possible, we felt that merely sawing the neck at the site of the desired fracture would provide a more definitive test of the fixation with no interlocking of fragments at the fracture site which could provide further unmeasurable stability.

Our set-up and machine was the same as that used by Hirsch and Brodetti [2] (Fig. 1). Strain gauges were used in their experiments to provide values of deflection which were slight. We used the same set-up and machine but no strain gauges. We used a revolving drum attached to the moving head of our compression apparatus. An ink writer provided us with curves. The abscissa



Fig 1

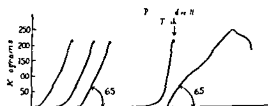


Fig 2 Curve obtained by pressure on the head superiorly

showing the distance head of femur moved (or deflected) while the ordinate showed the amount of force applied An ink writer traced curves which showed some similarity grossly although the final breaking point varied markedly in those specimens forced that far

Tests were done in three different positions Placing the bone upright with pressure on the head placing the bone with the trochanter resting on a block and the neck horizontal with the shaft gripped in a vise devised by Sig Bachman [1] A few specimens were held horizontally in Bachman s vise grip and the force applied to the anterior or posterior aspect of the head with support provided under the peritrochanteric line

The curves obtained by graphically recording deflection compared to force are shown in Figures 2 3 and 4 We applied the force more than once to the



Fig 3 Curve obtained when pressure applied posteriorly

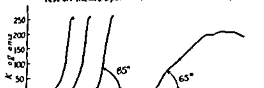


Fig 4 Curve obtained with pressure applied inferiorly

intact specimens in order to be sure the specimens were seated firmly in the vise grip. We accepted 3 similar curves on the same intact specimens as being indicative that the setup was correct and firm. Of course, the force could be applied only once to a nailed specimen.

The angle of ascent of the curve was measured by a simple protractor. Comparisons of angles of the curve were made at 50 kg. (120 lbs.) and 100 kg. (240 lbs.). The distance that the nailed head moved was also measured. X-rays were taken of specimens before and after nailing.

The curves of the deflection occurring with increasing force seemed to follow the same pattern for all the specimens. This was an initial rapid deflection for a few millimeters. When force was initially applied perhaps this reflected the specimen settling firmly in the vise grip or else the metal parts taking up slack. Then a graph represented an almost straight line up to the breaking point. In this series of experiments we carried the force to 250 kg. (well past body weights). The curves varied between 65° and 80°. Why these values varied we cannot say. There seemed to be no correlation to age or type of bone being tested. All of the graphs rose to an angle between 65° and 80° except one, #251 where the pressure was applied on the anterior aspect of the head. The curve here was 60°; perhaps there was some slight slipping of the specimen in the vise grip.

RESULTS

The more closely the graph of the nailed specimen approached the graph obtained from the same specimen when tested intact, the better was the nailing. Since we are comparing only a graph obtained from a nailed specimen with the graph from the same intact specimen, all extraneous factors such as age, degree of osteoporosis, and even variations in the machine do not enter into the comparison.

We grouped our results according to the figure obtained by adding the ratio of the angle of nailed specimen at 50 kg. to the angle of the intact specimen and the ratio of the angle of the nailed specimen at 100 kg. to the angle of the intact specimen. If the angles were all the same, in other words if the nailed specimen had the same strength and therefore the same amount of deflection as an intact specimen, each ratio would be 1 and the best result would have a value of 2. Where the deflection of the nailed specimen was greater this value decreased. We grouped our results into 3 groups. Those with values from 2 to 1.70 in Group 1, the best internal fixation. Group 2 values from 1.56 to 1.24 and Group 3 from 1.16 to .24, the internal fixation showing least strength.

In Group 1 the best results, all the specimens had the internal fixation device resting on a portion of the cortex opposite the area where the force was applied. The fragments moved relatively small distances. There were two exceptions, #11R was nailed with a long nail in the line of weight bearing, and it seemed to have good strength.

Group 2 with less stability of the internally fixed fragments showed the placement of nails more in the trabecular portion of the bone. Five were on the calcar but 2 of them were fractured in the shaft during fixation.

Group 3 had the poorest value. Almost uniformly the fixation was very poor or the fixation device rested on the midneck with little or no supporting structure around it. A few specimens were disrupted by a force below 100 kg. and one specimen was disrupted by a force below 50 kg.

DISCUSSION

Weight bearing with force applied to the superior aspect of the head of the femur is most commonly seen but other forces occur particularly abduction of the leg or adduction of the leg in bed. For this reason testing was done in different positions.

Several interesting occurrences took place when multiple small nails were



Fig 5

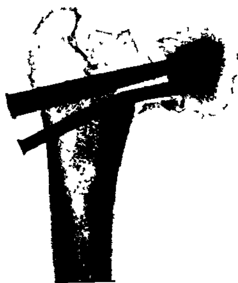


Fig 6



Fig 7



Fig 8

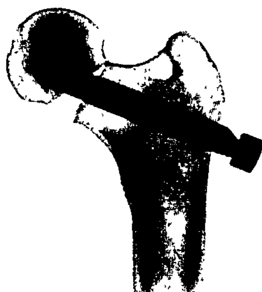


Fig. 9.

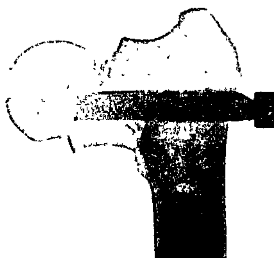


Fig. 10.



Fig. 11.



Fig. 12.

used, we found as shown in 13R 1 nail (Fig. 5, 6) resting on the calcar might bend while others in the medullary cavity merely slid. Perhaps 2 nails inferiorly on the calcar would provide greater strength. In 19L (Fig. 7) and 17R (Fig. 8) we found a long nail bent in mid position. Perhaps a long nail running through the trabeculae may have sufficient support to hold well. However, those areas where the trabeculae are thick and give support would have to be determined. Figure 9 shows a nail relatively far from the calcar (#13L). Yet this showed the calcar breaking as a unit (Fig. 10). Where do the trabeculae and cortex form a sufficiently strong area to support a nail?

Very rarely did the head move on the nail unless the nail were eccentrically placed or the specimen cracked in nailing. When screw type of internal fixation was used, we found that the head crashed down on the fixation device,

#6R (Fig 11 12) Perhaps some rotation took place or perhaps long flanges of the screw destroyed the trabeculae and permitted compression of bony substance within the head

Certainly this work must be correlated with the clinical findings in cases where nonunion of internally fixed femoral neck fractures occurred Perhaps repeated application of lesser forces simulating partial weight bearing as in crutch walking could cause different types of mechanical failure

CONCLUSIONS

A test has been devised to demonstrate mechanical stability of internal fixation of simulated neck fractures in autopsy bone disregarding such factors as age of patient amount of osteoporosis or breaking point of the individual specimens

Comparisons of different types of fixation can be done by this test

From the limited number of specimens and the random types of fixation it would seem that position of nail in relation to cortex was of more significance than type of fixation tested in this group

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POLYURETHANE POLYMER (OSTAMER) ITS USE IN FRACTURED AND DISEASED BONES *

Experimental Results

MICHAEL P MANDARINO AND JOSEPH E SALVATORE

Considerable interest in the past five years has been shown by several groups of investigators [1 2 3 4] in an attempt to obtain a suitable synthetic or plastic that could be poured in liquid form set within a reasonable length of time and have the qualities of strength osteogenicity nontoxicity cohesiveness and ease of handling required during surgery

Many types of plastics have been used safely for a wide variety of medical purposes, which suggested the application of such substances to the problem of osseous lesions Of the several possible materials which approach ideal requirements rigid polyurethane foam (Ostamer) is our compound of choice

For the purpose of producing a dense rigid cellular foam for orthopedic use a polymer is prepared by reacting a trihydroxy resin with an excess of

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diisocyanate. This polymer is mixed at the time of surgery with a catalyst. Carbon dioxide is liberated, producing a sponge like compound with 7 to 10% cellular structure. The polymer and the catalyst are in liquid form, and when mixed in a 1:1 ratio, result in a volume approximately three times that of the original. No heat is liberated.

It is apparent chemically that the polymer bonds to the surfaces to be held, bridged, filled or strengthened. The ostamer, in and during its formation, becomes an intimate part of the bone. It is this property that differentiates it from a glue, adhesive, or common cement-type bonding.

The rigid polyurethane form (ostamer), which is considered the best for orthopedic use at this time tests as follows: 1) bond strength to bone—from 170 to 190 lbs per square inch, 2) tensile strength—800 lbs per square inch, 3) compressive strength—20,000 lbs per square inch with 10% deflection; 4) density—25 to 30 lbs per cubic foot; 5) shear strength—500 lbs. per square inch, 6) flexural strength—1000 to 1200 lbs per square inch.

The original work was performed at Hahnemann Medical College and Hospital, Philadelphia, and has since been enhanced by the Army Institute of Research at Walter Reed Army Medical Center. The rather dramatic possibilities of the method are illustrated by the following case:

Case Report. Male age 29. Acute oblique fracture right distal tibia and fibula. Operation performed on 10-29-56. A reverse inlay graft was removed and the intermedullary canal was curetted. Two stainless steel pins were placed in the intermedullary trough and the medullary canal was filled with ostamer. The inlay graft was reversed, inserted and maintained by two Lowman clamps. The chemical was allowed to expand and set. The wound was closed. A posterior plaster splint was applied. On 11-1-56 three days postoperatively, the splint was removed, and x rays were taken with the patient in the supine position, then in a position of full weight bearing on the right leg. The patient was bearing full weight without crutches in 2 weeks. In 6 weeks x rays showed progressive healing. X rays taken 15 months postoperatively revealed complete healing.

METHOD

However, the purpose of this presentation is to describe some of the basic investigations which preceded its use in the human subject.

First, several pieces of bone bank bone were immersed in the basic toluene isocyanate. Negative toxic results on the bone fragments were observed microscopically. Rigid polyurethane molds were then placed in solutions—first water, then citrated blood, then whole blood—to determine dissolution, absorption and porosity of the plastic. In some 'pours' too much porosity and hydrophyllic action were noted, the mold becoming soft and spongy. Here is a danger in the use of this form, and for this reason exact measurements of ostamer plus catalyst are essential. A small mistake can result in a spongy, soft foam, ineffective for orthopedic use.

Industry's tests on guinea pigs were found to have produced nontoxic results. Guinea pig intraperitoneal innoculations performed by the authors were also negative for toxicity.

The use of ostamer is best restricted to those anatomical regions where adequate hemostasis by tourniquet or other established technique, may be secured. Sufficient fixation for wound closure occurs in 20 to 30 minutes. Complete hardening requires 18 to 24 hours, after which "curing" time, stability of the bonded area is such that weight bearing may be initiated.

Animal experimentation was then begun It consists of a systematic evaluation of the material This task is divided into three phases A) toxicologic, B) metabolic pathways and C) orthopedic

The toxicologic phase is extremely important because the polyurethane plastic is a high molecular weight polymer, and similar substances have been known to cause cellular aberrations in certain strains of rodents

To evaluate the ability of polyurethane to induce changes, three directions are being followed histologic sectioning hematologic studies and tissue culture studies

The histologic aspect of the study is being evaluated in rats and dogs The polyurethane plastic was implanted subcutaneously in 70 rodents The specimens that have been observed to date (7 months following implantation) show only minimal foreign body reactions and no evidence of carcinogenesis

The metabolic phase is an important aspect of the investigation since the polyurethane diminishes in volume over a period of time It is imperative that the metabolic route be known Is the material excreted or stored? What is the material s ultimate fate? These and many other questions are being studied The method of labeling with isotopes is very complex, and one of several methods is feasible The two most likely are tritium incubation of the final powder and/or specific labeling on the basic chemicals At present investigation is going on to select the most feasible

The orthopedic phase of the project is divided into animal and clinical groups The animal portion consists of approximately 66 dogs on whom 110 operations have been performed These dogs have been operated under nembutal anesthesia A defect in the radius or femur is created This defect is filled with plastic which bonds the bone ends together From previous



Fig 1 Dogs femur immediately after operation



Fig 2 Dogs femur 6 months after operation

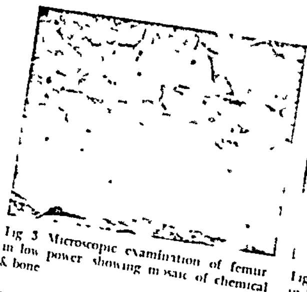


Fig 3 Microscopic examination of femur in low power showing mosaic of chemical & bone



Fig 4 Microscopic examination of femur in high power showing definite osteoblastic activity

experimentation it is known that the plastic slowly dissolves, permitting the bone to grow through the defect Varied results have been obtained in dogs, ranging from complete bridging of the defect with bone in 80% of the cases to no attempt of the bone to bridge the defect in the remaining 20% In the successful animals a cast has not been necessary Antibiotics are given routinely postoperatively

The hematologic portion is being studied by monthly complete blood counts, differential blood counts blood urea nitrogen determinations, and serum proteins from dogs with imbedded polyurethane plastic No alteration has been noted to date

Tissue culture studies using human cells are being carried out, and reports are now available, relating that there is an initial inhibition of cell growth which is rapidly resolved and that within a few days the cellular growth is normal, indicating no acute toxicity

Encouraged by the results in our animal experimentation, it was felt that suitable well chosen cases should be treated by this method of fixation Application of a polyurethane polymer (ostamer) for fixation of bone in 6 human beings has been used One acute fracture of tibia, 3 nonunions (1 of tibia, 1 supracondylar femoral), and 2 pathologic fractures of femurs were united by ostamer. Results in all cases have been satisfactory to date The ostamer was well tolerated by the hosts, and the strength of fixation was most gratifying to the surgeon

Extensive studies in toxicity, pharmacology, pathology, and carcinogenicity are being pursued

SUMMARY

A new material (polyurethane foam, rigid type, called ostamer) is being evaluated for use as an orthopedic device Results to date have been encouraging Full evaluation regarding toxicity and feasibility is being investigated

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FREEZE DRIED BONE HOMOGRAFTS *

Simon M K Myint, William T Fitts Jr Brooke Roberts
Jacques Jenny, Herndon B Lehr and Lewis K Anderson

The destruction of bone by trauma neoplasm or infection often necessitates the repair of large bony defects We have previously reported [1] on fresh circumferential homografts and autografts fixed with an intramedullary nail to replace defects in the dog s femoral shaft The homografts proved to be as successful as the autografts When a technique was developed that would solidly fix the graft to the host fragments both homografts and autografts were successful in about 75% of instances

We performed the experiments reported herein to determine if freeze dried bone grafts could be successfully substituted for fresh grafts The practical advantages of procuring storing and transporting freeze dried grafts of this type are apparent especially in military surgery

METHOD

Mongrel dogs weighing from 6 to 15 kg were observed for a period of 3 weeks before being subjected to the experimental procedure During this period each dog was given two 20 cc injections of homologous canine anti distemper serum and a 5 day course of oral sulfamerazine 1 5 gm daily After operation all dogs were given 100 000 units of penicillin intramuscularly daily for 5 consecutive days

Bone grafts used in these experiments were obtained under sterile conditions from the femoral and tibial shafts of healthy mongrel dogs at the end of acute experiments in the dog laboratory This bone was then frozen by placing it in a dry ice compartment with the temperature at minus 78°C It was kept at this temperature until it was freeze dried Four lots of bone were freeze dried The frozen bone segments were put in wide mouth test tubes and these were placed in the drying compartment of a freeze dryer An immediate vacuum was obtained and the bones were dried until a sample of bone placed in a vacuum desiccator containing phosphorus pentoxide did not lose more weight One lot of bone was freeze dried for 2 weeks at a pressure of 150 μ Hg The remaining three lots were freeze dried at separate times for 7 days each at a pressure of less than 50 μ Hg which proved to be sufficient time for the drying process The freeze dryer will accommodate only 15 tubes at a time hence the separation into lots There will be slight variations in the process for each lot The samples from all 4 lots were vacuum sealed in glass tubes and stored for 1 week to 6 months before being used The apparatus used for freeze drying this bone has been previously described

Circumferential bone segments from 1 5 cm to 2 cm long were cut under aseptic conditions from the freeze dried bone and inserted into an equal defect in the femoral shaft of the host dog A three phlanged intramedullary nail was inserted through the greater trochanter and down the medullary canal to fix the graft in place

* From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Supported by Contract No DA 49 007 MD 189 between the Department of the Army and the University of Pennsylvania

No external method of immobilization was applied, and the dogs were allowed to bear weight as soon as they were able. Healing of the grafts was studied by clinical examination, by roentgen examination at intervals, and by gross and microscopic examination at death or sacrifice of the animals

Freeze dried grafts were inserted into defects in the femoral shafts of 41 dogs. Grafts from Lot 1 were inserted into 10 dogs, from Lot 2 into 11 dogs, from Lot 3 into 12 dogs, and from Lot 4 into 8 dogs

RESULTS

Of the 10 dogs in Lot 1, 4 showed satisfactory healing of the graft (Fig. 1a, b, c) Three dogs died before firm bony union was achieved but in these 3 dogs early union was present. In 1 dog complete absorption of the graft occurred The 2 remaining dogs died of infection in the early postoperative period and were discarded. In Lot 1 there was only 1 definite failure of the graft. This lot was more successful than the other lots. It should be noted that in Lot 1 the conditions of freeze drying were different than in Lots 2, 3 and 4

For Lots 2, 3, and 4, the results were not as good as in Lot 1. Of the 31 dogs from these 3 lots, grafts were completely successful in 9 dogs. There were 15

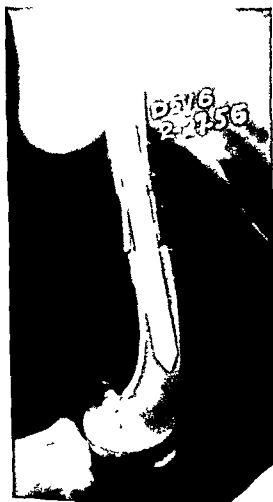


Fig 1a Freeze dried bone graft fixed with an intramedullary nail Roentgenogram immediately after nailing

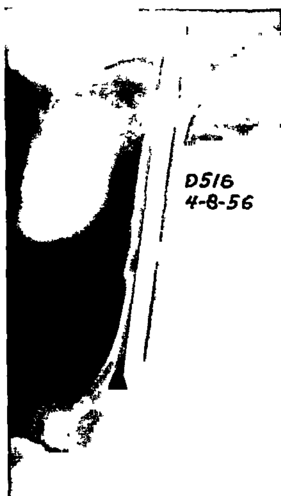


Fig 1b Roentgenogram 5 weeks after nailing Early bony union

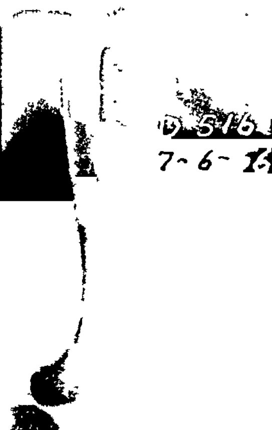


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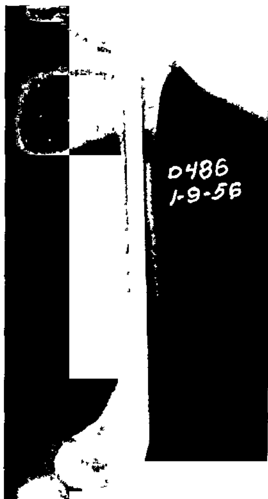


Fig. 2a Roentgenogram of freeze dried graft immediately after fixation with an intramedullary nail

graft failures. Ten dogs died of infection of the leg or unrelated disease in the early postoperative period. A typical graft failure is shown in Fig 2a, b, c.

DISCUSSION

The technique of freeze drying will probably influence the results obtained, but if all grafts are dried to a standard dryness, the effect of variations in the technique should be minimal. The rate of freezing and the time allowed for reconstitution of the grafts before being used will probably influence the results to a greater extent. These latter factors are being evaluated in continuing experiments and will be given in a subsequent report of these experiments

CONCLUSIONS

Under the conditions of these experiments, freeze-dried circumferential bone grafts were not as successful as fresh homografts and fresh autografts However, in view of a number of successful freeze-dried grafts obtained under these conditions of severe stress on the grafts, and in view of the ease with which freeze-dried bone can be stockpiled, this method should be further evaluated.

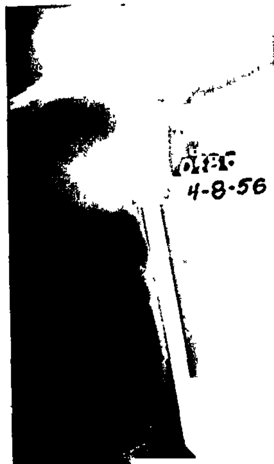


Fig 2b Roentgenogram 3 months after nailing



Fig 2c Roentgenogram 4 months after nailing showing further absorption of the graft.

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of the specimens from each of 5 selected animals (4, 6 15 and 24 weeks after the procedure utilizing internal fixation and 24 weeks after the procedure without the utilization of internal fixation) are illustrated (Fig 4, 5)

Following the encouraging results of this group we then operated upon another group of 10 dogs in the same fashion, half of them with internal fixation and half without Four were sacrificed in 9 months and 6 at 1 year We felt that the experimental work could be safely terminated at 1 year since if adequate bone formation had not developed in this length of time the procedure would not be worth while as applied clinically

Of the animals sacrificed at 9 months the 2 with the fixation device showed bony union of the adjoining vertebral bodies (Fig 6, 7) The 9 month controls had developed a more advanced synarthrosis The observations on the 6 twelve month animals yielded similar results (Fig 6 7) the three with the device showing good bony union

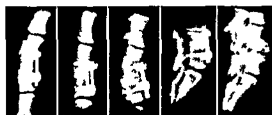


Fig 4 A panorama of the lateral view roentgenograms of the necropsy specimens arranged in chronologic order and showing the progression of bony union From left to right 4 week 6 week 15 week and 24 week specimens and 24 week control specimen

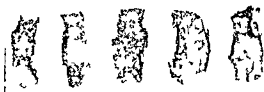


Fig 5 A panorama of the photomicrographs of the necropsy specimens arranged in chronologic order From left to right 4 week 6 week 15 week and 24 week specimens and 24 week control specimen Hematoxylin eosin—methylene blue × 3½



Fig 6 Lateral view roentgenograms of necropsy specimens from animals sacrificed 9 months and 12 months after operation Controls (on left) show absence of bony union and synarthrosis formation on right both specimens show bony union (clamp removed from 12 month specimen)

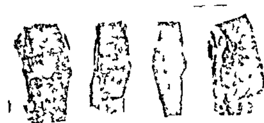


Fig 7 Photomicrographs of necropsy specimens show the formation of a synarthrosis in the controls at both 9 and 12 months bony union complete in the 9 and 12 month specimens utilizing clamps Hematoxylin eosin—methylene, × 3½

In none of the animals with the clamp has there been any evidence of pseudarthrosis formation This technique fulfills all 4 postulates for bony union

CONCLUSION

A method of anterior spinal fusion is presented It has resulted in solid bone union in all instances in which the procedure was followed In those experimental animals where fixation was omitted synarthrosis occurred in each instance, indicating that adequate immobilization is essential for the consistent production of bone fusion between vertebral bodies

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THE EXPERIMENTAL USE OF NOVOBIOCIN (CATHOMYCIN) AND OTHER ANTIBIOTICS IN THE TREATMENT OF EXPERIMENTALLY PRODUCED PURULENT ARTHRITIS *

Charles J Frankel, A Brant Lipscomb and David K Webster

Pyogenic arthritis including pyogenic arthritis developing as a result of direct penetration of the joint by the hematogenous route or by direct extension from the metaphyseal region has been treated by the use of penicillin both intraarticularly and parenterally The drug has been used as an adjunct with aspiration irrigation and at times incision and drainage

In 1945 Burns Young and Muller [1] reported 101 penetrating wounds of the knee joint affecting World War II casualties These injuries were treated by the intraarticular injection and by the parenteral use of penicillin 2 to 8 days following the injury They reported 75 knees developed a full range of pain free motion 15 knees had better than 90° of motion and 11 had ankylosis Cultures were rendered sterile in each case by the treatment In 1952 Altemeier and Largen [2] reported 70 cases of pyogenic arthritis treated with intraarticular and parenteral penicillin parenteral chloramphenicol aureomycin and terramycin Twenty two cases were hematogenous in origin and the offending organism was the hemolytic staphylococcus Twenty six other cases were due to gonococcus and 22 to a variety of organisms including the hemolytic streptococcus pneumococcus meningococcus B paracolon, and H influenzae Eighty nine per cent excellent or good results were reported and in only one case was incision and drainage necessary

Jocson [3] in 1955 studied the diffusion of antibiotics through the synovial membrane in humans and found that aqueous potassium penicillin G was the only antibiotic which when administered parenterally was able to cross

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the synovial barrier in therapeutic amounts He also found that procaine penicillin streptomycin, aureomycin terramycin, and chloramphenicol did not cross the synovial barrier in amounts sufficient to be of therapeutic value The author concluded that the treatment of choice in cases of pyogenic arthritis due to penicillin sensitive organisms was aqueous penicillin (potassium G) and that the other drugs used in the test were of value only in preventing spread of the infection into the blood stream

In spite of Jocson's provocative report and in spite of the ever increasing evidence of penicillin resistant staphylococcus infections, there have been no reports regarding the intraarticular use of the broad spectrum antibiotics During the past few years we have carried on experimental work in which the knee joints of adult rabbits were infected and treated under aseptic conditions by the use of the intraarticular injection of novobiocin

METHOD

Two rabbits were injected daily for 5 days with a saline solution containing 25 mg and 50 mg novobiocin respectively The animals were sacrificed at 14 days and studies were made of the synovial tissue and the joints to determine the effect, if any, of the drug on normal tissues

The knee joints of 4 rabbits were injected with a 1 cc solution containing 90,000,000 penicillin resistant coagulase positive staphylococcus aureus organisms sensitive to novobiocin These animals were sacrificed at 14 days

Four rabbits were infected with 1 cc solution containing 90,000 000 penicillin resistant coagulase positive staphylococcus [illegible] after infection, daily intraarticular injections o[illegible] biocin respectively were given for 5 days The [illegible] days It was not feasible to aspirate and irrigate the joints prior to the instillation of novobiocin

Four rabbits were infected with 1 cc of the same organism as used in the other animals Forty eight hours after injection, daily injections of 25 mg and 50 mg of novobiocin were begun, intravenously for 5 days Three of the animals died on the fifth day and the fourth died on the sixth day

No other parenteral administration of the drug was utilized

RESULTS

After injection of the noninfected joint with novobiocin there was no gross effusion Microscopically, there was minimal edema and injection of the synovium The cartilage appeared intact, there was some fibroplasia of the synovium, and capillaries together with chronic inflammatory cell infiltration

After injection of 1 cc of the organism severe joint destruction occurred A thick purulent exudate, a thickened and injected synovium, and almost complete cartilage destruction were observed Microscopically, there was necrosis, marked thickening of the synovium with fibroplasia, capillary ingrowth and acute and chronic inflammatory cells were observed There was complete destruction of the cartilage Culture at the time of sacrifice was, of course, positive

After treatment of the infected joints with novobiocin moderate synovial thickening and injection were seen grossly There was minimal sero purulent effusion The cartilage was intact Microscopically, moderate synovial thickening with fibroplasia, capillary ingrowth, and chronic inflammatory cell

infiltration was observed. As expected, this degree of inflammatory reaction was considerably greater than in the knees where novobiocin alone was injected and in which no infection had been introduced. However, the cartilage remained intact. No necrosis or acute inflammatory cells were present. The cultures were negative in all instances.

SUMMARY AND CONCLUSIONS

1. The intraarticular use of novobiocin in rabbits is an effective method of handling joint infection due to penicillin resistant coagulase positive staphylococcus aureus.
2. Novobiocin is soluble in saline and when injected does not seriously irritate or damage the normal synovia or the cartilage.
3. The intravenous use of novobiocin was ineffective. The material was either too toxic for intravenous use or did not succeed in reaching the infection.
4. The broad spectrum antibiotics we believe do not effectively cross the synovial barrier. Their use in joint infections is valuable only when the drugs are introduced locally into the joints.
5. Three human patients were treated by repeated aspiration, irrigation, and installation of novobiocin solutions. Excellent results have been obtained.
6. Similar studies have been carried out on a number of rabbits in which erythromycin, kanamycin, and bacitracin were used, intraarticularly. These results will be reported later.

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THE EFFECT OF ENVIRONMENTAL INFLUENCE ON ACETABULAR DEVELOPMENT *

CARL R. COLEMAN, RICHARD F. SLAGER, AND WILLIAM S. SMITH

The present report represents one phase of experimental and clinical studies on the etiology of congenital dislocation of the hip. In a previous report before the Orthopedic Research Society,[1] it was demonstrated that pronounced acetabular dysplasia and torsional changes in the femoral head and neck could be produced by experimental dislocation of a weight bearing ball and socket joint in puppies.

The next logical problem was to determine how alteration of the local environment of the acetabulum could influence its development. Normally, the

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femoral head within the socket constitutes the immediate environment of the acetabulum Of what significance is the shape of the femoral head in the development of the acetabulum? An experiment was devised to determine the effects of other geometric patterns placed within the acetabulum in growing puppies

METHOD

Twenty five puppies with an average age of 4 weeks were used In 1 group of 7 animals the right femoral head and neck were resected and well fitting titanium spheres were inserted in the acetabulum (Fig 1) The spheres were secured in position by silk sutures to the cartilaginous limbus A second group, consisting of 13 puppies underwent resection of the femoral head and neck, followed by insertion of titanium cubes (Fig 2) In a third group of 5 puppies, the capital femoral epiphysis was detached from the neck of the femur at the epiphyseal plate by sharp dissection (Fig 3) The ligamentum teres was not disturbed The capsule was closed around the superiorly dislocated femoral neck

Of the animals in which titanium spheres were substituted for femoral heads, 5 were suitable for study at the time of maturity Following dissection, the spherical contour of the acetabulum was seen to conform exactly to the mold applied The soft tissue was then digested by warming in a solution of 5% sodium hydroxide In all cases there was a thick osseous rim of the anterior, superior, and posterior margins of the acetabulum, The depth of the acetabulum was well maintained (Fig 4)

Seven titanium cubes remained within the acetabulum until the time of sacrifice Soft tissue digestion again showed the thickened osseous rim of the anterior superior, and inferior acetabulum However, the marginal bone was more abundant following application of the cubes than in the cases of the

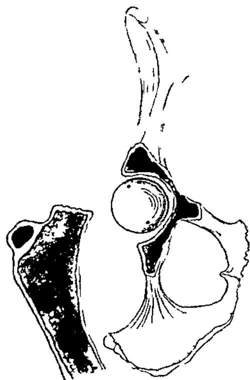


Fig 1 Substitution of a titanium sphere for resected femoral head

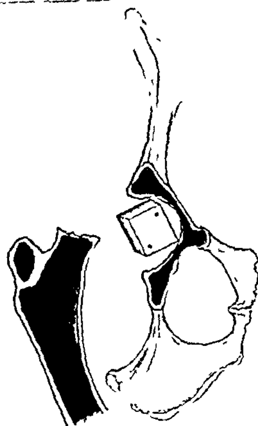


Fig 2 Substitution of a titanium cube for resected femoral head

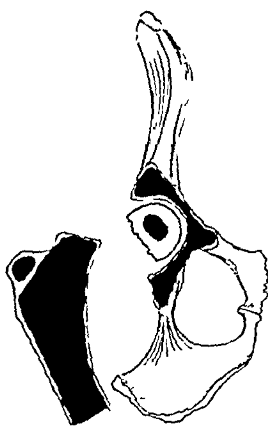


Fig 3 Surgically produced epiphyseolysis

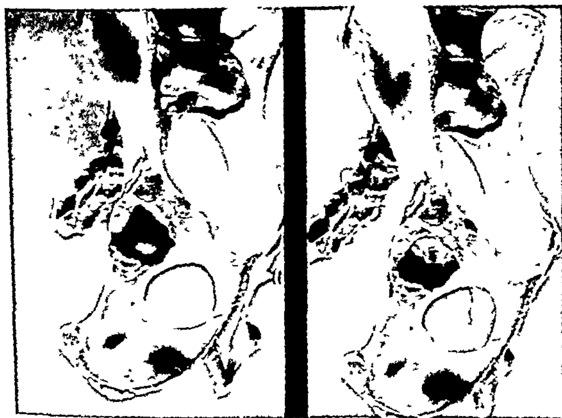


Fig 4 Dry specimen at maturity following substitution of a titanium sphere for the femoral head (see text)

spheres (Fig 5) The depth was not as well maintained as the cases of the spheres however the floor of the acetabulum was flattened rather than concave as with the spheres

Four animals with capital femoral epiphysiolysis were followed to maturity The acetabula in this group resembled the normal more than any of the previous groups The depth was less than the normal side the size was smaller and the acetabular margin was only slightly thickened

Objections to the experimental approach to this problem are not without recognition The hardness of the metal the method of securing the implants and the foreign body nature of the implants must be considered Certain patterns however, cannot be denied The maintenance of depth and hypertrophied rim must be regarded as expressions of the functional demand placed upon the acetabulum by its contents When we consider the acetabular aplasia resulting from the absence of the femoral head in the socket soon after birth the dependence of acetabular development on local environment is evident

The Relationship of Early Alteration of the Acetabulum to Acetabular Development, Subluxation, and Dislocation Primary hypoplasia of the roof of the acetabulum has gained increased prominence as it applies to congenital dislocation of the hip The theory of preluxation of the hip leading to dislocation presupposes a hypoplastic superior acetabular rim Whether hypoplasia is an inherited defect or whether it is secondary to a mechanical phenomena in the birth canal is debatable

To test the importance of the cartilaginous limbus as it relates to the

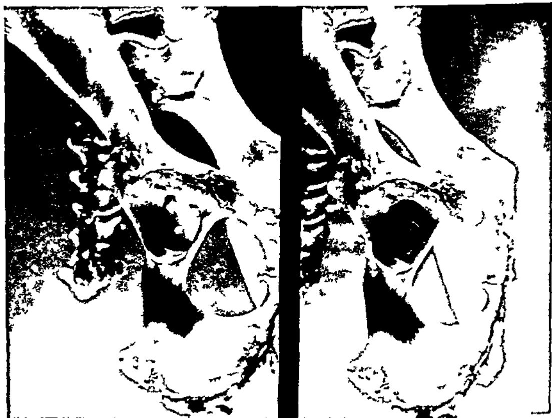


Fig 5 Dry specimen at maturity following substitution of titanium cube for femoral head (see text)

development and the integrity of the hip joint, the entire anterior, superior, and posterior cartilaginous portions of the acetabulum were resected in 16 puppies 4 weeks after birth. In 10 of the animals, the ligamentum teres was not disturbed, but in 6 animals it was transected.

One month after resection of the limbus and sparing of the ligamentum teres, roentgenograms showed what was to be expected. There was increased acetabular obliquity in all specimens and what would be clinically interpreted as subluxation. However, at the conclusion of 6 months, none of the hips was dislocated. All were still clinically subluxated, but the acetabulum was well developed and somewhat "shallow" by clinical standards, despite the fact that the animals were ambulatory immediately after recovering from anesthesia (Fig 6).

The combination of resection of the limbus plus transection of the ligamentum teres resulted in complete dislocation in 2 of the 6 animals in this group (Fig. 7) The picture of the remaining 4 animals could hardly be distinguished from the previous group at the time of maturity.

In this phase of the study the real question is whether or not a surgically produced shallow socket in an experimental animal is comparable to a socket which is hypoplastic either from embryonic development or secondary atrophy from pressure in utero Since dislocation can occur under these circumstances when the ligamentum teres is transected, a predislocation phase of congenital dislocation of the hip cannot be excluded on an experimental basis.

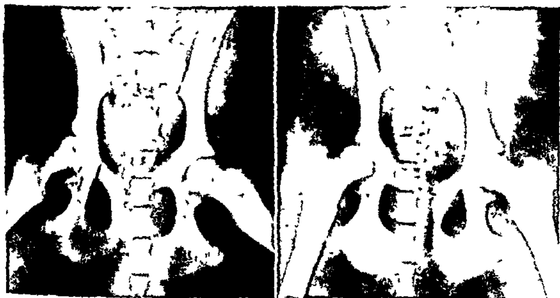


Fig 6 Five months after resection of the limbus in 2 animals Although there is some acetabular obliquity subluxation has not progressed to dislocation

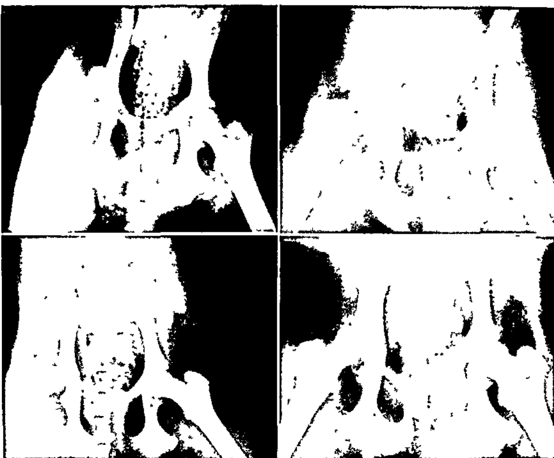


Fig 7. (top) Mature dogs illustrating dislocation following resection of the limbus and section of the ligamentum teres
(bottom) Mature dogs following resection of the limbus and section of the ligamentum teres Dislocation has not taken place.

CONCLUSIONS

1. The development of the acetabulum is dependent upon the geometric pattern within it during the growth phase.

2 Alteration of the acetabulum by surgically producing a shallow socket in dogs soon after birth does not result in disclocation of the hip but merely results in a shallow socket and what is clinically referred to as subluxation

3 Alteration of the acetabulum in dogs soon after birth by a surgically produced shallow socket plus section of the ligamentum teres may result in disclocation during the subsequent growth phase.

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Plastic Surgery

THE BEHAVIOR OF SKIN GRAFTS EXCHANGED BETWEEN PARENTS AND OFFSPRING *

LYNDON A. PEER AND JOHN C. WALKER, JR.

The material presented in this study describes the behavior of specific tolerated skin homografts some 12 months after the last published article on the subject. In general, the skin homografts exchanged between mother and infant or child, which were reported as tolerated one year ago, have not been rejected as of this time. Those which demonstrated only relative tolerance have been gradually replaced by host skin (Fig. 1). We have also extended the scope of our work using skin tolerance or relative tolerance as a test for tolerance to cartilage homografts in children with congenital absence of an external ear. In two cases children who tolerated their mother's skin have also tolerated their mother's rib cartilage for 12 months without gross evidence of absorption.

METHOD

Our experimental work began with the successful transfer of skin from a mother to a 3 months old male infant who had received, at the age of 3 weeks, an intramuscular injection of 2 ml. of maternal blood [1] The infant had been followed with biopsy and electrophoresis studies, and had not rejected the skin graft of the mother by 210 days. At this time the graft was removed and microscopic examination demonstrated normal epidermis, but there was a very dense infiltration of lymphocytes within the dermis and an absence of hairs and glands. The adjacent host skin was entirely free of

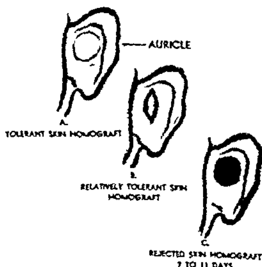


Fig 1 Skin grafts exchanged between mothers and children may demonstrate early rejection as an acute phenomenon, or may be relatively tolerant showing considerable contracture, pigmentation and superficial bleb formation Occasional homoskin grafts appear grossly to be tolerated like autografts for long periods of time

* From the St Barnabas Rehabilitation Center, Newark, N. J This work was made possible by a grant from the John A Hartford Foundation, Inc.

this lymphocytic infiltration Epidermal cells in the homograft showed the sex chromatin which is characteristic of female skin[2] (Fig 2)

The injection procedure was followed on the assumption that pretreatment with cells from a prospective skin donor might create a state of tolerance in young infants similar to that observed in the experimental work of Medawar, *Billingham, and Sparrow*[3] *These authors injected fetal mice or chicks with* living homogenous cells and thereby created tolerance of skin grafts from donors that had provided the inoculum Using another species (rats) Woodruff[4] injected the young on the day of birth with splenic breis and noted that these newborn rats were tolerant in later life to skin grafts from the rat that provided the injection Applying this principle to humans Woodruff,[5] in 2 cases, injected living leucocytes from fathers into the thigh muscles of their respective infants 18 and 3 hours after birth When the infants were 6 months old, each received a split graft from its father Both grafts were initial takes and persisted until 4 weeks but thereafter decreased considerably in size

Injected Infants. Eighteen additional infants of both sexes have been injected intramuscularly by our group[6] with 4 ml of whole blood taken from parents with compatible blood groups (Blood groups were determined only as to O, A, B, AB, and Rh factors) The age of the infants at the time of injection varied from 3 to 28 days, and the time interval between injection and subsequent skin transplantation ranged from 40 to 75 days Full thickness skin grafts were interchanged between parents and infants in 17 cases In the remaining one case the transplantation was only from parent to infant Four exchanges were with fathers and the remaining 13 with mothers

Dark discoloration or mottling of the graft was considered the end point of survival Two grafts from infants to mothers developed superficial blebs in the central quarter but both healed, apparently by proliferation of graft epithelium these were evaluated as successful and are still in place 97 and 99 days respectively following transplantation (Fig 3, A,B,C) Prolonged survival of the grafts on infants or parents did not appear to be related to the sex of the child or its age at the time of blood injection, the time interval between blood injection and skin exchange, or to be dependent on any fixed pattern of blood groups We found, however, that all prolonged survivals

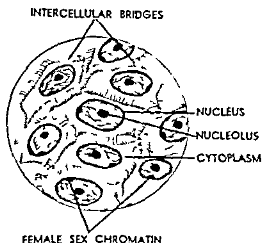


Fig 2 Female epidermal cells showing characteristic female sex chromatin body in contact with nuclear membrane Sex chromatin study affords a positive method for determining the survival of homogenous skin grafts when the donor and recipient are members of opposite sexes The method may also be useful to determine the survival of cells in cartilage and corneal homografts

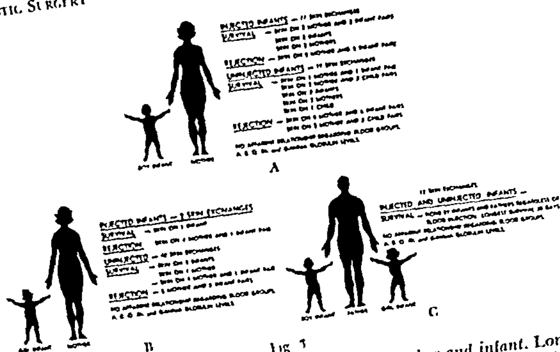


Fig. 3

occurred *when the grafts were exchanged between mother and infant*. Long-surviving grafts from mother to infant have persisted for 820 days. A rather surprising finding, not anticipated, was the still longer survival time of the child's skin behind the mother's ear, these homografts having remained for as long as 36 months.

The grafts exchanged between fathers and infants *were all rejected both ways*. The longest survival of father skin on infants was 11 days. The longest survival of infant skin on fathers was 20 days. The survival times of grafts interchanged between mother and child had no apparent correlation. Some mothers retained their child's skin long after the child had rejected the mother's skin, and *vice versa*. The gamma globulin levels of the infants varied, but long surviving skin homografts occurred in children with levels of 7%, 15.2% and 8.3% of the total proteins, as determined by electrophoresis.

Uninjected Infants. A control series of 33 skin interchanges between parents and infants or parents and older children with compatible blood groups, the offspring not having received a blood injection from the parent, has yielded some interesting findings.[6] A survey demonstrated no prolonged survival when the exchange was made *between father and male or female infant*. Prolonged survival (up to 361 and 496 days) has been obtained when the exchange was made *between mother and male or female infant* (Fig. 1, A,B). In 2 cases exchanges of skin were made between mothers and their 7 and 12 year old boy children. These homotransplants have been retained by both pairs for 90 and 150 days respectively.

Biopsy study of the homograft from mother to 12 year old boy 3 months after transplantation demonstrated the sex chromatin in the epidermal cells which is characteristic of female skin.[2] Biopsy of the boy's skin graft on the mother† showed an absence of female sex chromatin. The transplant, however, *did not appear like an autograft*. The epidermal layer was normal but there was an absence of hairs and glands, and the dermis was infiltrated

† This skin graft from boy child to mother is still surviving 467 days following transplantation. The mother's skin on the boy was gradually replaced by 5 months following transplantation.





A B

Fig 4 Mother, boy infant exchange 213 days after transplantation. The infant did not receive a blood injection and was grafted when 2 months old. *A*, mother's skin behind infant's ear shows relative tolerance having undergone considerable contracture, pigmentation and thinning Superficial blebs appeared in the center of this graft 40 days after transplantation but these later completely healed *B*, child's skin behind mother's ear has been well tolerated.

by a dense collection of lymphocytes. Small blood vessels in the dermis contained normal-appearing blood cells.

CONCLUSIONS

1. Long survival of homografts (30 days or longer) occurred only when the skin was exchanged between mother and offspring. About 25% of the homografts exchanged between mothers and their boy or girl children were long survivals. Seventy-five per cent were rejected within 20 days after transplantation.

2. The longer survival of skin homografts from mother to child than from father to child suggest that tolerance between mother and child may occur because of fetal exposure to maternal substances (antigens?). This might lead to "acquired tolerance" in the sense of Billingham, *et al.*[7] The early rejection of grafts from mother to child may possibly be explained by a failure of fetal exchange to take place, for reasons unknown. It appears evident from these experiments that the prolonged survival of skin grafts exchanged between mother and infant or child was not due to the blood injection The blood injection may have been administered to the infant during a "null period", when exposure to an antigenic stimulus has no appreciable effect[7] The extremely long survival of the child's skin on the mother is difficult to explain A possible reason for the relative tolerance to father's skin (6 weeks) reported by Woodruff[5] may be owing to the fact

that he injected younger infants (18 and 3 hours old) with a much larger amount of leucocytes There may however be some dangers associated with the injection of large numbers of leucocytes in newborn infants and we agree with Woodruff that this should be clarified by further animal experimental work

3 The prolonged survival time of some skin grafts from mother to child suggest the possible clinical value of blood transfusions from mother to child rather than from fathers or unrelated donors Our preliminary findings also suggest the advisability of using mothers as donors of skin homografts for severely burned children

4 Sex chromatin study of the epidermal cells in homoskin transplants offers a positive method for determining the survival of the graft when the exchange is made between male and female

5 Children who tolerate their mother s skin *may also tolerate other tissues from their mother* such as kidney and endocrine glands Routine skin exchanges may prove to be useful tests for tolerance to other tissues from mother to child and *vice versa*

6 The long survival of skin grafts exchanged between mothers and 7 and 12 year old children indicates that tolerance persists as the infant grows older

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THE ALTERED RESPONSE TO SKIN HOMOGRAFTS AND TO DELAYED ALLERGENS IN HODGKIN S DISEASE *

WILLIAM D KELLY ROBERT A GOOD RICHARD L VARCO AND MICHAEL LEVITT

It has long been known that Hodgkin s disease is commonly associated with tuberculosis and certain other chronic fungus diseases[1 2] In the case of tuberculosis this circumstance has been explained as related to the occurrence of anergy to tuberculin which has frequently been noted[3] Recently Schier *et al*[4] reported a high incidence of anergy in patients with Hodgkin s disease skin tested for delayed hypersensitivity with a variety of antigens prepared from commonly encountered microorganisms Since the phenomenon of homograft rejection in man and other animals is generally classified as resembling most closely the reactions termed delayed hyper sensitivity[5] the occurrence of anergy to delayed allergens in Hodgkin s disease offers the opportunity to put to the test the importance of this type

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of immune responsiveness to homograft rejection in man as well as delineate a new potential approach to the etiology and treatment of this puzzling disease

METHOD

Skin Testing for Delayed Hypersensitivity. Thirty four patients with Hodgkin's disease and 208 patients with a variety of diseases excluding malignancy in general were skin tested by the intradermal injection of the forearms with 0 1 ml of solutions or suspensions of the following antigens 1) Schick control solution (diphtheria toxoid containing 008 lf), 2) streptokinase streptodornase (10 U S K, 2 5 U S D), 3) mumps skin testing antigen (Lederle), 4) mumps skin testing control solution (Lederle), 5) trichophyton gypseum extract, 1 to 30 dilution, 6) Candida albicans extract 1 to 100 dilution, 7) P P D, intermediate strength (0 0001 mg) Patients receiving steroids or being treated with nitrogen mustard or similar compounds were not tested until at least 1 week after discontinuance of therapy None of the patients tested was critically ill or moribund Readings were made of the skin reactions shortly after application and at 24 and 48 hours Erythema and induration were measured in two diameters at right angles to each other A positive reaction is taken to mean the occurrence of induration and the presence of erythema and/or induration totaling 5 mm or more in diameter Ages ranged from 11 to 69 years in the Hodgkin's disease group and from 21 to 81 years in the control group All patients were male except for 3 female children with Hodgkin's disease In addition to the above groups a moderate number of skin tests as described were applied to patients with the following diseases lymphoma other than Hodgkin's disease, leukemia, carcinoma, and malnutrition

Induction of Hypersensitivity. Nine patients with Hodgkin's disease and 14 patients with a variety of other diseases were tested for their ability to develop delayed hypersensitivity to diphtheria toxoid using a specific precipitate of diphtheria toxin antitoxin injected intradermally as described by Uhr *et al* [6] These patients were all demonstrated to be negative to diphtheria *toxoid skin testing previously Two weeks after the intradermal injection of* 0 5 ml of a suspension of the specific precipitate the patients were retested for delayed hypersensitivity to diphtheria toxoid

A small number of patients with Hodgkin's disease was studied for the following items serum gamma globulins, typhoid paratyphoid agglutinin formation, mumps complement fixation antibody formation, polio neutralizing antibody formation, isoagglutinin titer The methods have been previously described [7]

Fifteen patients with Hodgkin's disease were further studied by the performance of a skin homograft Selection of these cases depended largely on willingness to participate voluntarily in the study and the anticipated ease of prolonged frequent follow up All of these patients were ambulatory None of them was receiving steroids or radiomimetic compounds at the time of grafting although some of them were receiving irradiation to localized areas distant from the area of the graft Under local anesthesia a 1 5 by 2 5 cm diamond shaped full thickness piece of skin was excised, usually from the anterior thigh, and replaced with a full thickness homograft of the same dimensions which was tattooed with India ink in a regular pattern to aid in identification during subsequent follow up Stitches were removed after 7

to 10 days following which the fate of the graft was determined by frequent inspection and palpation and with photographic recording

RESULTS

The findings are summarized in Tables 1 to 5 It is noteworthy that 5 of the patients with Hodgkin s disease tested for the induction of delayed hypersensitivity with specific precipitate (Table 4) had previously shown at least 1 positive response to 1 of the 6 other antigens used in the initial battery other than the diphtheria toxoid The initial take was complete or nearly complete in all homografts The subsequent behavior of the skin grafts fell into 3 categories Two patients tolerated the graft as if it were an autograft Both patients were completely anergic to the battery of delayed allergens These cases were followed for 7 and 15 months after placement A second group of 10 patients appeared to accept the grafts without reaction for a period of 3 to 4 weeks following which a reaction would appear characterized by the formation of a red crust and/or blistering which would involve a variable area of the graft Erythema was noted in the surrounding host skin In many instances it was thought that complete rejection was occurring However, after several weeks the crusts would separate revealing intact epidermis beneath which the tattooed pattern of the original graft was still apparent to a greater or lesser degree In some instances this pattern of reaction recurred after a short interval of time In 2 grafts (both children) the end result was slough of the entire graft at approximately 3 months after placement In the remaining cases in this group the reaction subsided with

Table 1 Frequency of Positive Responses to Delayed Allergens in Hodgkin s Disease and Controls

	SCHICK CONTROL	SK SD	MUMPS	MUMPS CONTROL	TRICHOPHYTON	CANDIDA	PPD
Hodgkin s Patients (34)	7%	21%	32%	0%	21%	9%	15%
Non Hodgkin s Patients (208)	10%	66%	88%	3%	61%	57%	52%

Table 2 Frequency Distribution of Positive Responses to Delayed Allergens in Hodgkin s Disease and Controls

NO POSITIVE RESPONSES	HODGKIN S PATIENTS (34)	NON HODGKIN S PATIENTS (208)
0	20 (59%)	3 (1%)
1	4 (12%)	8 (4%)
2	6 (18%)	41 (20%)
3	1 (3%)	59 (28%)
4	1 (3%)	60 (29%)
5	2 (5%)	30 (15%)
6	0	5 (2%)
7	0	2 (1%)

of immune responsiveness to homograft rejection in man as well as delineate a new potential approach to the etiology and treatment of this puzzling disease.

METHOD

Skin Testing for Delayed Hypersensitivity. Thirty-four patients with Hodgkin's disease and 208 patients with a variety of diseases excluding malignancy in general were skin tested by the intradermal injection of the forearms with 0.1 ml. of solutions or suspensions of the following antigens: 1) Schick control solution (diphtheria toxoid containing .008 Lf); 2) streptokinase-streptodornase (10 U.S.K., 2.5 U.S.D.); 3) mumps skin testing antigen (Lederle); 4) mumps skin testing control solution (Lederle); 5) trichophyton gypseum extract, 1 to 30 dilution; 6) Candida albicans extract, 1 to 100 dilution; 7) P.P.D., intermediate strength (0.0001 mg.). Patients receiving steroids or being treated with nitrogen mustard or similar compounds were not tested until at least 1 week after discontinuance of therapy. None of the patients tested was critically ill or moribund. Readings were made of the skin reactions shortly after application and at 24 and 48 hours. Erythema and induration were measured in two diameters at right angles to each other. A positive reaction is taken to mean the occurrence of induration and the presence of erythema and/or induration totaling 5 mm. or more in diameter. Ages ranged from 11 to 69 years in the Hodgkin's disease group and from 21 to 81 years in the control group. All patients were male except for 3 female children with Hodgkin's disease. In addition to the above groups a moderate number of skin tests as described were applied to patients with the following diseases: lymphoma other than Hodgkin's disease, leukemia, carcinoma, and malnutrition.

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A small number of patients with Hodgkin's disease was studied for the following items: serum gamma globulins, typhoid-paratyphoid agglutinin formation, mumps complement fixation antibody formation, polio neutralizing antibody formation, isoagglutinin titer. The methods have been previously described.[7]

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The findings are summarized in Tables 1 to 5. It is noteworthy that 5 of the patients with Hodgkin's disease tested for the induction of delayed hypersensitivity with specific precipitate (Table 1) had previously shown at least 1 positive response to 1 of the 6 other antigens used in the initial battery other than the diphtheria toxoid. The initial take was complete or nearly complete in all homografts. The subsequent behavior of the skin grafts fell into 3 categories. Two patients tolerated the graft as if it were an autograft. Both patients were completely anergic to the battery of delayed allergens. These cases were followed for 7 and 15 months after placement. A second group of 10 patients appeared to accept the grafts without reaction for a period of 3 to 4 weeks following which a reaction would appear characterized by the formation of a red crust and/or blistering which would involve a variable area of the graft. Erythema was noted in the surrounding host skin. In many instances it was thought that complete rejection was occurring. However, after several weeks the crusts would separate revealing intact epidermis beneath which the tattooed pattern of the original graft was still apparent to a greater or lesser degree. In some instances this pattern of reaction recurred after a short interval of time. In 2 grafts (both children) the end result was slough of the entire graft at approximately 3 months after placement. In the remaining cases in this group the reaction subsided with

Table 1. Frequency of Positive Responses to Delayed Allergens in Hodgkin's Disease and Controls

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Table 2. Frequency Distribution of Positive Responses to Delayed Allergens in Hodgkin's Disease and Controls

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5	2 (5%)	30 (15%)
6	0	5 (2%)
7	0	2 (1%)

Table 3 Incidence of Anergy to Delayed Allergens in Various Diseases

	NO ANERGIC	NO TESTED
Hodgkin s Disease	20	34
Lymphoma (excluding Hodgkin s disease)	3	13
Leukemia	4	17
Carcinoma	3	38
Malnutrition	0	4

Table 4 Production of Delayed Hypersensitivity to Diphtheria Toxoid

	NO POSITIVE	NO TESTED
Hodgkin s Disease	0	9
Controls	9	14

Table 5 Immunological Evaluation of Patients with Hodgkin's Disease

	NO PRESENT	NO TESTED
1 Normal or increased serum gamma globulin	8	8
2 Typhoid paratyphoid agglutinin formation	7	7
3 Mumps complement fixation antibody formation	4	4
4 Isoagglutinin titers normal	5	5
5 Polio neutralizing antibody formation	0	4

what appears to be at least a portion of the original graft remaining No subsequent reaction occurred in grafts of this variety followed as long as 13 months Four patients in this group showed 1 or 2 positive responses when skin tested with the battery of delayed allergens In a third group of 3 patients the grafts underwent early rapid necrosis with subsequent complete slough in the manner usually seen with homografts in man Two of these patients showed 1 and 4 positive responses respectively to the battery of delayed allergens

DISCUSSION

The occurrence of a high incidence of anergy to delayed allergens as reported by Schier *et al*[4] is confirmed by the present work Moreover, the preliminary observations employing a specific precipitate to induce hyper sensitivity suggest that Hodgkin's disease patients are deficient in this respect even though they may show a positive reaction to a battery of delayed al lergens prepared from commonly encountered microorganisms If this is verified by further study, it will help explain the apparent discrepancy

resulting from the fact some of the patients with a positive skin response when tested with the battery of delayed allergens subsequently showed partial tolerance to a skin homograft. The preliminary study of serum antibodies and response to immunization are interpreted as indicating no striking deficiency in this respect in Hodgkin's disease. The usual fate of homografts in man is sudden slough beginning around 10 days after placement.[8] Prolonged survival of skin homografts in man has been reported in patients with extensive burns[9] and uremia.[10] None of these cases showed graft survival beyond 6 weeks, however, in contradistinction to the present report. The interpretation of prolonged graft survival in those cases in the present report in which a delayed reaction occurred rests largely on the persistence of at least a part of the original pattern of tattooed India ink dots in the dermis. The possibility that gradual replacement of the graft elements with host tissue could occur without disturbance of this pattern is considered unlikely but not impossible. Moreover, it is conceded that in these cases only the dermis may be retained with loss of epidermis and host replacement. This question is currently being investigated. The behavior of skin homografts placed on patients with Hodgkin's disease supports the concept that immune responses of the delayed hypersensitivity variety are involved in homograft rejection in man. That this is not the sole factor, however, is seen in the fact that skin homografts have been reported to survive for indefinite periods of time in patients with congenital aggammaglobulinemia, in which condition the induction of delayed hypersensitivity can be demonstrated whereas the formation of serum antibodies is deficient.[11]

SUMMARY

Skin tests for delayed hypersensitivity using solution of antigens from a variety of commonly encountered microorganisms indicate that patients with Hodgkin's disease show a high incidence of anergy in contrast to control patients. Preliminary observations suggest that Hodgkin's patients are deficient in their ability to develop a new delayed hypersensitivity in response to an effective antigen but are not deficient in serum gamma globulins, isoagglutinins, or the ability to form serum antibodies in response to conventional immunization techniques. Full thickness homografts placed on Hodgkin's disease patients resulted in early rejection in 3, complete acceptance in 2, and what is interpreted as delayed reaction resulting in slough in 2 and partial indefinite survival in 8. These results support the concept that immune reactions of the delayed hypersensitivity variety are involved (but not solely) in homograft rejection in man.

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STUDIES ON MAMMALIAN HOMOTRANSPLANTS OF SKIN *

II Tolerance Induced with a Pooled Antigen of White Blood Cells and Ribonucleic Acid

FRANKLIN L ASHLEY, SHARON A MOONEY, JOHN W EDWARDS, EUGENE N GARCIA, JOHN M SYVERUD AND MIGUEL ALONSO ARTIEDA

Extensive investigations have been reported in the contemporary literature involving the use of foreign cells as antigens to enhance the acceptance of homotransplants of skin in animals and chickens This phenomenon, called "tolerance," has been described by Billingham, Brent and Medawar,[1] Ashley,[2] Cannon, *et al*,[3] and Hasek [4]

In an earlier study by the present authors,[5] it was found that a pooled antigen of whole blood or its component parts could be used to create tolerance in 1) noninbred related rats, 2) noninbred nonrelated rats, and 3) noninbred, nonrelated rats in which concentrated leucocytes were used as antigen, and 4) further, it was found that in 66 rats 30 to 50% accepted grafts following the injection of a pooled antigen of concentrated white blood cells from 10 nonrelated donors of a group of animals entirely separate from and nonrelated to the original donor group Those animals receiving the pooled antigens displayed significant differences in percentage take and duration of take of the homotransplants from the controls (maintained for each group) It was further found that with an increase in the age at the time of grafting, there was a decrease in the animals ability to accept the transplant This held true whether the donor was related or non related and also applied to the controls (the latter, using an equal number of animals to the injected group, revealed only 20% survival after 24 days and 12 6% survival after 8 months, including both related and non related animals)

METHOD

The animals used for experimentation were nonrelated rats of the Long Evans, Sprague Dawley, and Wistar strains

Dorsal suprapannicular grafts, approximately 3 by 5 cm, were exchanged and rotated To secure the grafts, six 11 mm Michel clips were used All grafts were observed grossly to determine onset of slough Histopathologic examinations confirmed the gross observations of the grafts' condition at various times

Experiment 1 (Group E). Thirty eight 1 to 3 day old neonatal rats were injected with a pooled, concentrated, white blood cell antigen derived from 20 nonrelated, young adult donors Five hundredths cubic centimeter (approximately 2,500,000 white blood cells) was administered by intracardiac injection to each animal The animals were then switch grafted at 21 days of age using nonrelated donors of different strains but of the same age as the recipient

Experiment 2 (Group RNA). Forty six 1 to 3 day old neonatal rats were injected intracardially with a dilute solution (1 mg/1 cc of normal saline)

* From the Department of Surgery University of California Medical Center Los Angeles
Supported in part by contract with the Division of Medical Research and Development United States Army No DA-49 007 MD 616

of ribonucleic acid (RNA) which was obtained from rat livers and spleens after the method of Colter and Brown.[6] Five hundredths cubic centimeter was given to each animal. The animals were then switch grafted at 21 days of age, again using nonrelated donors of a different strain but of the same age as the recipient. These donors were not related to the donors of the livers and spleens used as the source of the RNA, however, they were of the same strain.

DISCUSSION AND RESULTS

In Experiment 1, our results seemed to confirm those of an earlier study, although too soon to be conclusive (38 animals: 15% survival up to 10 days). These animals were all switch grafted at 21 days of age. We plan a similar study for the future in which the grafts will be exchanged at 25, 30, 35, and 40 days of age. Due to previous failures above this level, we feel it unnecessary to repeat grafting in animals over 15 days of age.

In Experiment 2 (Group RNA), a dilute solution of ribonucleic acid obtained by our biochemist, Mr. Gene Garcia, was injected as the antigen to attempt the creation of tolerance. These injections were given intracardially on the first to third neonatal days. Interestingly enough, there was a 35% survival after 18 days in a total of 16 animals grafted. This work was influenced by the report of Billingham, *et al.*[7] in which it was pointed out that cells totally disintegrated by ultrasonic irradiation retain their power to elicit skin transplantation immunity in mice. It was also felt that the substances responsible for this phenomenon reside wholly within the nucleus and are developed before birth. They are inactivated by digestion with desoxyribonuclease but not with ribonuclease or trypsin. Our preliminary results would seem to agree in part with this but not with the portion involving ribonuclease. More complete results will be forthcoming.

As mentioned previously, the animals in Experiment 1 were switch grafted. Following this procedure, we were surprised to note that 38% of the grafts transferred from the injected animals to the graft donor animals survived 18 days (to date). This also applied to the RNA animals in the same percentage. In the latter group, however, the percentage survival of the grafts applied to the donor animals was higher (38%) after 10 days than the percentage survival (35%) after 18 days of the grafts applied to the animals previously injected with RNA. This may indicate that the graft itself plays some part in the tolerance phenomenon. Heretofore, the general concensus of opinion has been that the tolerance mechanism is induced by an adaptation of the host and not the graft.[8] While our results are entirely of a preliminary nature, the implication is intriguing. We shall be most interested to follow the progress of the grafts and report more conclusive results in the future.

The controls from our former study were used for comparison in these two groups and additional numbers of control animals are now under observation. It appears however, even in a preliminary way, that there will be an equally significant difference between the percentage take among the injected animals and the new controls (35 to 15%: 8 to 10%).

SUMMARY

Two groups of animals have been studied with respect to the creation of tolerance following the injection of (1) a pooled antigen and (2) a purified

ribonucleic acid These results have been compared with the results and controls of an earlier study

It was found in Experiment 1 that following the injection of a pooled antigen of concentrated white blood cells from 20 nonrelated donors, 45% of the 38 rats challenged accepted skin homotransplants from another group of animals of the same age and of a different strain than the original antigen donor group

In Experiment 2, ribonucleic acid prepared from livers and spleens of one strain of rat was then used as antigen to attempt the creation of tolerance in another group of rats of a different strain It was found that there was a 35% survival of these grafts after 48 days in 46 animals grafted The animals receiving the antigen in both experiments were switch grafted with the graft donor animals There was a 38% survival of these grafts on the donor animals after 48 days in the first experiment and 38% survival after 40 days in the second experiment

Significant differences (percentage survival) were noted between the animals in both experimental groups and the control animals 35 to 45% 8 to 10%

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ACQUIRED TOLERANCE FOR HOMOLOGOUS TRANSPLANTATION OF NORMAL TISSUES AND ORGANS *

CARLOS MARTINEZ, JUNE M SMITH, J BRADLEY AUST AND ROBERT A GOOD

Acquired tolerance, allowing homotransplantation of normal and neoplastic tissues has been induced by injecting the prospective recipient animals with living cells from the future donor during embryonic life or shortly after birth [1 2 3 4 9]

In this report data are presented on the induction of tolerance to homologous transplantation of skin and some endocrine glands, such as ovaries

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and pituitaries in mice of different strains using several donor host strain combinations

METHOD

Mice of the A Ce Z (C3H) CBA BALB/C C57 Bl (subline 1) and ZCe F1 and ZBC hybrids were used Acquired tolerance was induced by the method recommended by Billingham and Brent[2] consisting of the intra venous injection of viable spleen cells into newborn animals not later than 24 hours after birth The preparation of the spleen cell suspension and the technique for the intravenous injection have been described elsewhere[6] At 45 to 60 days of age injected animals were submitted to homotrans plantation of skin ovaries or pituitaries taken from adult animals isologous with the spleen donors

Tolerance to Skin In this experiment several donor host combinations were used Z (C3H)→Ce Ce→Z (C3H) ZBC→Ce Ce→ZBC A→Z (C3H) Z (C3H)→A A→ZCe F1 CBA→ZCe F1 BALB/C→ZCe F1 and C57 Bl→ ZCe F1

A piece of full thickness abdominal skin of approximately 1 5 by 2 0 cm in size taken from the donor was transferred to the back of the recipient as previously described[6] After grafting mice were kept under observation for a period of at least 2 months

The results are listed in Table 1 Of 10 Ce mice treated at birth with Z (C3H) cells all accepted homologous Z (C3H) skin Of 13 animals of the same kind (Ce) treated with ZBC cells 9 or 69% were susceptible to ZBC skin Most of the ZBC animals treated at birth with Ce spleen cells were subsequently tolerant to this skin (94%) It is interesting to note that of 17 Z (C3H) mice receiving A spleen cells at birth only 5 or 28% were tolerant to this skin However all mice of the same stock Z (C3H) similarly treated with Ce cells became tolerant to Ce skin On the other hand A strain mice treated with Z (C3H) cells failed in all instances to accept Z (C3H) skin Finally both groups of ZCe F1 hybrids pretreated with either CBA or C57 Bl strain cells accepted their homologous skin in all instances In contra distinction similar groups of ZCe F1 treated with A tissue accepted A skin in a rather small proportion (26%) whereas the same hybrids pretreated with BALB/C uniformly failed to accept this skin

Table 1 Acquired Tolerance to Skin Homografts in Different Strains of Mice

HOST STRAIN	DONOR STRAIN	NUMBER OF MICE ACCEPTING SKIN HOMOGRAFT	PER CENT	HOMOGRAFT CONTROLS	PER CENT
Ce	Z(C3H)	10/10	100	0/15	0
Ce	ZBC	9/13	69	0/15	0
ZBC	Ce	33/35	94	0/66	0
Z(C3H)	A	5/17	28	0/12	0
Z(C3H)	Ce	12/12	100	0/10	0
A	Z(C3H)	0/10	0	0/12	0
ZCe F1	A	5/19	26	0/8	0
ZCe F	CBA	11/11	100	0/8	0
ZCe F1	BALB/C	0/9	0	0/14	0
ZCe F1	C57 Bl	4/4	100	0/7	0

Attempts to graft skin between homologous control individuals of these strains and corresponding hybrids were regularly unsuccessful

Tolerance to Ovaries. In this experiment the following donor host strain combinations were used BALB/C→ZCe F_1, Ce→ZBC, Ce→Z(C3H) and Z(C3H)→Ce At approximately 60 days of age injected mice were submitted to bilateral ovariectomy followed immediately by transplantation of 2 ovaries taken from female mice of the strain corresponding to that from which the spleen cells were derived Donors were of the same age as the recipients

Starting at 10 to 15 days following surgery vaginal smears were taken from each animal to determine the presence or absence of estrous cycles This was done at intervals of 10 days and samples were taken daily for 4 consecutive days

Two to 3 months after ovarian transplantation the grafts were removed for histological study, and further daily observations of the vaginal changes were continued throughout a period of 15 days

Ovariectomized mice not treated at birth with homologous spleen were also transplanted with homologous ovaries and served as controls

The results are shown in Table 2 Of 4 mice of the ZCe F_1 hybrid strain treated at birth with BALB/C cells, 2 accepted BALB/C ovaries Of 19 mice of the ZBC stock pretreated with Ce spleen, all accepted Ce ovaries Similar results were obtained in 5 mice of the Z(C3H) strain pretreated with Z(C3H) strain cells

All animals accepting homologous ovaries exhibited normal sexual function for as long as the grafts were left in place (2 to 3 months) Upon removal of the grafts the sexual activity ceased

As it would be expected all 12 castrate ZBC control females failed to accept Ce ovaries as reflected by the absence of ovarian function Similarly,

Table 2 Ovarian Homotransplantation in Tolerant Mice

HOST STRAIN †	DONOR STRAIN	NUMBER OF MICE WITH OVARIAN HOMOGRAFTS	NUMBER OF MICE SHOWING NORMAL ESTROUS CYCLES	PER CENT
ZCe F_1	BALB/C	4	2	50
ZBC	Ce	19	19	100
Z(C3H)	Ce	5	5	100
Ce	Z(C3H)	6	6	100
Total		34	32	94
		Controls ††		
ZBC	Ce	12	0	0
ZCe F_1	BALB/C	14	0	0
Total		26	0	0

† Female mice pretreated with homologous spleen cells intravenously at birth castrated at 2 months of age and transplanted with ovaries from the donor strain originally providing the spleen cells

†† Female mice castrated at 2 months of age and transplanted with ovaries from the donor strain indicated

uniform failures occurred in 11 control ZCe F_1 hybrid mice transplanted with BALB/C strain ovaries

Tolerance to Hypophysis. In this experiment female mice of the following donor host strain combinations were used: CBA→ZCe F_1; C57 Bl→ZCe F_1; BALB/CA F_1→ZCe F_1; and Ce→ZBC At approximately 2 months following the spleen cell injection recipient animals were submitted to total hypophysectomy by the technique described by Thomas[7] This was followed at the same setting by the transfer of the pituitary graft into the emptied "sella turcica" as previously described[8] Donors of the graft were mice of the same strain as those used for spleen injections and of the same sex and age as that of the host.

The success of the hypophyseal graft was judged by 1) the ability of the host to increase its body weight and 2) the presence of a normal sexual function as indicated by the study of the estrous cycles

Groups of ZBC and ZCe F_1 hybrid female mice not injected at birth with homologous spleen cells received intracellular hypophyseal homografts taken from Ce and BALB/CA F_1 donors respectively and served as controls

The results are summarized in Table 3 All 3 mice of the ZCe F_1 hybrid stock treated at birth with CBA spleen cells accepted hypophyseal homografts following hypophysectomy as judged by the subsequent increase in body weight and the presence of normal estrous cycles On the other hand, of the group of ZCe F_1 mice pretreated with C57 Bl spleen, 1 out of 3 accepted C57 Bl pituitaries and 1 out of 2 of the same strain (ZCe F_1) treated with BALB/CA F_1 spleen accepted hypophyseal grafts from BALB/CA F_1 donors Finally, of the group of 12 ZBC mice receiving Ce spleen cells, 10 became tolerant and accepted homologous Ce pituitary grafts

None of the control mice accepted homologous transplants of hypophyseal glands as indicated by their failure to grow and the absence of estrous cycles Figure 1 compares the growth of hypophysectomized tolerant mice successfully homotransplanted with pituitary glands and the failure of growth in the nontolerant controls which were hypophysectomized and transplanted with homologous pituitary glands

Table 3 Hypophyseal Homografts in Tolerant and Nontolerant Mice

Host strain	Donor strain †	Number of mice grafted with homol hypoph	Number of mice accepting the graft ††	Per cent
ZCe F_1	CBA	3	3	100
ZCe F_1	C57 Bl	3	1	33
ZCe F_1	BALB/CA F	2	1	50
ZBC	Ce	12	10	83
Total		20	15	75
		Controls		
ZBC	Ce	6	0	0
ZCe F_1	CBA	10	0	0

† Spleen cells from this strain injected at birth

†† As judged by a progressive increase in body weight and cyclic vaginal activity

Attempts to graft skin between homologous control individuals of these strains and corresponding hybrids were regularly unsuccessful

Tolerance to Ovaries. *In this experiment the following donor host strain combinations were used* BALB/C→ZCe F_1, Ce→ZBC, Ce→Z (C3H) and Z (C3H)→Ce At approximately 60 days of age injected mice were submitted to bilateral ovariectomy followed immediately by transplantation of 2 ovaries taken from female mice of the strain corresponding to that from which the spleen cells were derived Donors were of the same age as the recipients

Starting at 10 to 15 days following surgery vaginal smears were taken from each animal to determine the presence or absence of estrous cycles This was done at intervals of 10 days and samples were taken daily for 4 consecutive days

Two to 3 months after ovarian transplantation the grafts were removed *for histological study, and further daily observations of the vaginal changes* were continued throughout a period of 15 days

Ovariectomized mice not treated at birth with homologous spleen were also transplanted with homologous ovaries and served as controls

The results are shown in Table 2 Of 4 mice of the ZCe F_1 hybrid strain treated at birth with BALB/C cells, 2 accepted BALB/C ovaries Of 19 mice of the ZBC stock pretreated with Ce spleen, all accepted Ce ovaries Similar results were obtained in 5 mice of the Z (C3H) strain pretreated with Z (C3H) strain cells

All animals accepting homologous ovaries exhibited normal sexual function for as long as the grafts were left in place (2 to 3 months) Upon removal of the grafts the sexual activity ceased

As it would be expected all 12 castrate ZBC control females failed to accept Ce ovaries as reflected by the absence of ovarian function Similarly,

Table 2 Ovarian Homotransplantation in Tolerant Mice

HOST STRAIN †	DONOR STRAIN	NUMBER OF MICE WITH OVARIAN HOMOGRAFTS	NUMBER OF MICE SHOWING NORMAL ESTROUS CYCLES	PER CENT
ZCe F_1	BALB/C	4	2	50
ZBC	Ce	19	19	100
Z(C3H)	Ce	5	5	100
Ce	Z(C3H)	6	6	100
Total		34	32	94
		Controls ††		
ZBC	Ce	12	0	0
ZCe F_1	BALB/C	14	0	0
Total		26	0	0

† Female mice pretreated with homologous spleen cells intravenously at birth castrated at 2 months of age and transplanted with ovaries from the donor strain originally providing the spleen cells

†† Female mice castrated at 2 months of age and transplanted with ovaries from the donor strain indicated

uniform failures occurred in 14 control ZCe F_1 hybrid mice transplanted with BALB/C strain ovaries.

Tolerance to Hypophysis. In this experiment female mice of the following donor host strain combinations were used: CBA→ZCe F_1; C57 Bl→ZCe F_1; BALB/CA F_1→ZCe F_1; and Ce→ZBC. At approximately 2 months following the spleen cell injection recipient animals were submitted to total hypophysectomy by the technique described by Thomas.[7] This was followed at the same setting by the transfer of the pituitary graft into the emptied "sella turcica" as previously described.[8] Donors of the graft were mice of the same strain as those used for spleen injections and of the same sex and age as that of the host.

The success of the hypophyseal graft was judged by: 1) the ability of the host to increase its body weight and 2) the presence of a normal sexual function as indicated by the study of the estrous cycles.

Groups of ZBC and ZCe F_1 hybrid female mice not injected at birth with homologous spleen cells received intracellar hypophyseal homografts taken from Ce and BALB/CA F_1 donors respectively and served as controls.

The results are summarized in Table 3. All 3 mice of the ZCe F_1 hybrid stock treated at birth with CBA spleen cells accepted hypophyseal homografts following hypophysectomy as judged by the subsequent increase in body weight and the presence of normal estrous cycles. On the other hand, of the group of ZCe F_1 mice pretreated with C57 Bl spleen, 1 out of 3 accepted C57 Bl pituitaries and 1 out of 2 of the same strain (ZCe F_1) treated with BALB/CA F_1 spleen accepted hypophyseal grafts from BALB/CA F_1 donors. Finally, of the group of 12 ZBC mice receiving Ce spleen cells, 10 became tolerant and accepted homologous Ce pituitary grafts.

None of the control mice accepted homologous transplants of hypophyseal glands as indicated by their failure to grow and the absence of estrous cycles. Figure 1 compares the growth of hypophysectomized tolerant mice successfully homotransplanted with pituitary glands and the failure of growth in the nontolerant controls which were hypophysectomized and transplanted with homologous pituitary glands.

Table 3. Hypophyseal Homografts in Tolerant and Nontolerant Mice

Host Strain	Donor Strain †	Number of Mice Grafted with Homol. Hypoph.	Number of Mice Accepting the Graft ††	Per Cent
ZCe F_1	CBA	3	3	100
ZCe F_1	C57 Bl	3	1	33
ZCe F_1	BALB/CA F_1	2	1	50
ZBC	Ce	12	10	83
Total		20	15	75
		Controls		
ZBC	Ce	6	0	0
ZCe F_1	CBA	10	0	0

† Spleen cells from this strain injected at birth.
†† As judged by a progressive increase in body weight and cyclic vaginal activity.

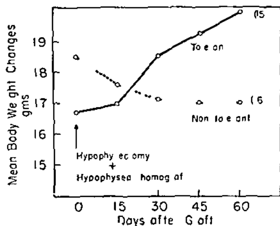


Fig 1 Mean body weight changes in tolerant (solid line) and nontolerant (broken line) hypophysectomized mice bearing hypophyseal homografts Between parentheses number of animals per group

DISCUSSION

The results obtained confirm and extend those of Billingham *et al* [1, 2, 5] indicating that acquired tolerance permitting homologous transplantation of skin and organs can be achieved in the mouse by exposing newborn animals to living spleen cells taken from the prospective homologous donors

In regard to the skin the incidence of successful homografts varied with the different donor host strain combination used In fact treatment at birth in several donor host strain combinations such as Z (C3H)→Ce ZBC→Ce Ce→ZBC Ce→Z (C3H) CBA→ZCe F_1 C57 Bl→Zce F_1 produced high incidences of tolerance while in other strain combinations such as A→Z (C3H) Z (C3H)→A A→ZCe F_1 and BALB/C→ZCe F_1 tolerance was induced in a small proportion of animals or not at all Furthermore while all ZCe F_1 treated with either CBA or C57 Bl spleen cells developed tolerance to these tissues few of the same hybrids became tolerant when the donor cells were derived from either A or BALB/C strain This might be interpreted as indicating the existence of strain differences in the capacity of the donor cells to induce tolerance when administered to newborn mice of a given strain

In regard to the results on acquired tolerance to ovaries and pituitaries they demonstrate that the transplanted glands can retain their functional activity while residing in tolerant individuals In fact tolerant mice bearing homologous ovaries showed normal estrual activity for as long as the grafts were left in place Similarly pituitary homografts placed in tolerant animals previously deprived of their own glands revealed successful establishment of the graft in a frequency comparable to that previously encountered in isografts [8] Hypophysectomized animals bearing hypophyseal grafts showed progressive increase in body weight and normal sexual activity In contradistinction ovaries and pituitaries transplanted across genetic barriers in nontolerant animals did not survive or function normally

These data then add consistency to the speculation advanced by Billingham *et al* [2] that the induction of acquired tolerance will allow transplantation of functional active glands or tissues other than the skin

SUMMARY

Acquired tolerance for homologous transplantation of skin and some endocrine glands such as ovaries and pituitaries has been induced by

intravenous injection of viable homologous spleen cells into newborn mice of several inbred strains and hybrids

Differences in susceptibility to induction of tolerance for homologous skin varied from strain to strain combination depending mainly on the donor strain of spleen cells used in newborn inoculation.

Homologous ovaries and pituitaries transplanted into tolerant animals retain their normal function as judged by persistence of cyclic vaginal changes in case of ovaries and sustained increase in body weight and normal sexual function in case of homotransplanted pituitaries.

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THE IMMUNE REACTION IN ADRENALECTOMIZED PATIENTS MAINTAINED ON METICORTEN, DEMONSTRATED BY SKIN HOMOGRAFT REJECTION *

Preliminary Investigation of the Possible Hypoallergenicity of Skin from Adrenalectomized Patients Maintained on Meticorten

CADVAN O GRIFFITHS, JR, M D, AND GEORGE F CRIKELAIR, M.D

It has been suggested by comparison to the immunological reactivity of foreign proteins that host intolerance to homografts is an antigen antibody reaction [1] The excitor of this reaction is postulated as being related to the antigenicity of cytoplasmic and perhaps nuclear protein complexes,[2, 3] and although no serum antibodies have as yet been demonstrated against these antigens, skin homograft rejection in other respects satisfies the criteria of an immune reaction In this latter regard it can be shown that skin homografts require a latent period for development of host reactivity and demonstrate the condition of anamnesis when they are reapplied after immunity has been established [4]

It is the purpose of this investigation to establish a possible normal deviation exhibited by adrenalectomized patients maintained on meticorten by

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examination of their immune response to transplanted skin, and to note the effects of steroid administration on the survival of their skin when transplanted to normal subjects

METHOD

One hospitalized and 3 employed, asymptomatic, bilaterally adrenalectomized patients, maintained on from 50 to 70 mg. of meticorten o.d. with advanced hormone dependent neoplastic disease, and 4 white male volunteers were chosen.

Baseline eosinophile counts using Swanson's[5] modification of Randolf's[6] technique and filter paper electrophoretic serum protein studies employing Osserman's[7] method, were obtained on all patients. Each was then subjected to the surgical excision under local 1% procaine anesthesia of four 2 by 3 cm. segments of skin of approximately ten thousandths of an inch in thickness. The wounds were disposed at least 10 cm. apart, one in each of the four abdominal quadrants. After removal of the skin 3 of the denuded areas were excised down to subcutaneous fat and the dermis discarded, bleeders being clamped and tied with 5-0 plain catgut. On 3 of these defects in each of the 8 patients, an autograft, a skin homograft from an adrenalectomized individual, and a homograft from a normal male volunteer were secured in place with interrupted sutures of 5-0 dermalon after india ink was tattooed into the Caucasian homografts for purposes of identification. The remaining defect was left denuded in 2 adrenalectomized patients and in 2 normal volunteers and excised down to subcutaneous fat in the other 4 subjects. Xeroform gauze and a dry sterile pressure dressing were applied over all operative areas.

Postoperatively, all wounds and grafts were inspected at regular intervals and redressed with xeroform gauze. Sutures from the grafts were removed on the seventh postoperative day.

Healing of open wounds was checked by inspection and biopsy. Autograft vascularization was evaluated by observing changes in the color of the graft after transplantation or by biopsy. The degree of contracture of the grafts was recorded by measuring each graft surface area once a week.

Homografts, after primary take had occurred, were observed for the earliest evidence of rejection by noting changes in their external characteristics. This rejection was most frequently manifested by the formation of a small ecchymotic area beneath the surface epithelium followed by epithelial denudation and serum exudation. Throughout the experiment biopsies were taken of both autografts and homografts to evaluate differences in elicited vascular and cellular response between control and adrenalectomized patients and serial eosinophile counts and filter paper protein electrophoretic studies were done to follow the possible systemic effects of homograft rejection.

RESULTS

Autografts in both groups of patients were well vascularized by the third or fourth day postgrafting and firmly attached to their beds by the sixth day. Contracture of all grafts occurred maximally during the first month, persisting to the third month, and amounting to not less than 30% nor more than 15% of the original surface area of the grafts. No dissimilarity in graft contracture of adrenalectomized patients and controls was apparent.

Homografts were indistinguishable from autografts by inspection and

biopsy until the first evidence of rejection appeared (Table 1). Inasmuch as biopsies of the homografts demonstrated an asynchronous rejection of epithelium throughout the graft with persistence of epithelial viability for periods in excess of 5 days following the initial appearance of rejection, and since homograft epithelium was not demonstrated to have been overgrown by host epithelium, the time required by the host to heal the homografted bed was selected as a relative end point of rejection (Table 2).

Table 1. Skin Homograft Rejection

NORMAL MALE VOLUNTEERS	ADRENALECTOMIZED PATIENTS
V 1[illegible]-1[illegible] Days (4 cases)	V 11-20 Days (3 cases)
V 9-11 Days (4 cases)	A 10-18 Days (5 cases)

V=Volunteer Homografts
A=Adrenalectomized Homografts

Table 2. Secondary Healing After Homograft Rejection

NORMAL MALE VOLUNTEERS	ADRENALECTOMIZED PATIENTS
V 2[illegible]-[illegible]7 Days (4 cases)	V 22-30 Days (5 cases)
A 20-23 Days (4 cases)	A 20-2[illegible] Days (5 cases)

V=Volunteer Homografts
A=Adrenalectomized Homografts

With the notable exception of one hospitalized female adrenalectomized patient, homograft rejection in adrenalectomized individuals maintained on meticorten followed the same pattern as the controls. Biopsies revealed no differences in the degree or kind of cellular or vascular reaction accompanying rejection of homografts, and secondary healing of the grafted sites after homograft rejection progressed without inhibition in both adrenalectomized and normal patients.

The homografts of the female adrenalectomized patient who demonstrated no external signs of rejection appeared moderately altered from their original state after a period of 6 months. Their surfaces had become glazed, their borders less distinct, their india ink markings less vivid and more diffuse, their color bluish gray, and their size decreased by almost 30%. Serial biopsies performed during the 6 month period showed minimal cellular reaction in the corium and subcutaneous tissues. In the one homograft transferred from a male donor it was possible to show complete replacement of epithelium and dermis by the host through reference to the nuclear sex chromosomal pattern of biopsy specimens (Fig. 1 and 2). In the remaining homograft transplanted from a female, one could only infer that it too had been replaced by noting the dispersal of its original india ink markings into the deep subcutaneous blood vessel endothelium of the host.

Serial serum electrophoretic protein patterns in adrenalectomized and control patients yielded no changes from those observed preoperatively. The moderate decrease in gamma globulin noted in 3 adrenalectomized patients did not alter the time sequence of homograft rejection in 2, but may have contributed to the nature of the rejection in the remaining one.

examination of their immune response to transplanted skin, and to note the effects of steroid administration on the survival of their skin when transplanted to normal subjects

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Table 1. Mean Homograft Rejection

Normal Male Volunteers	Adrenalectomized Patients
A [illegible] Days ([illegible] cases)	A 11-20 Days (3 cases)
N [illegible] Days ([illegible] cases)	N 10-18 Days (3 cases)

A = [illegible] Homografts
N = [illegible] Homografts

Table 2. Secondary Healing After Homograft Rejection

Normal Male Volunteers	Adrenalectomized Patients
A [illegible] Days ([illegible] cases)	A [illegible]-30 Days (3 cases)
N 20-25 Days ([illegible] cases)	N 20-[illegible] Days (3 cases)

A = [illegible] Homografts
N = [illegible] Homografts

With the notable exception of one hospitalized female adrenalectomized patient, homograft rejection in adrenalectomized individuals maintained on meticorten followed the same pattern as the controls. Biopsies revealed no differences in the degree or kind of cellular or vascular reaction accompanying rejection of homografts, and secondary healing of the grafted sites after homograft rejection progressed without inhibition in both adrenalectomized and normal patients.

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Fig 1 Photomicrograph of the epidermis of a male homograft transplanted to an adrenalectomized female patient which demonstrated no external signs of rejection Note the eccentric nuclear chromatin mass (arrow) on the nuclear membrane of one of the epithelial cells This is characteristic of cells found in the female sex and indicates that the original male homograft had been replaced by its female host



Fig 2 Photograph of the subepithelial stroma of the homograft in Figure 3 Again note the eccentric chromatin masses (arrows) indicating that the dermal elements in the original homograft had been similarly replaced

The eosinophile response to homograft rejection followed no definite pattern in the 8 patients in this investigation

DISCUSSION

The failure to demonstrate significant differences in survival time of homografts transplanted from normal volunteers to 3 of 4 adrenalectomized patients maintained on meticorten suggests that the immunological mechanisms of these adrenalectomized patients maintained on meticorten are functioning satisfactorily The fact that meticorten replacement in adrenalectomized patients does not affect the survival time of homotransplanted skin from adrenalectomized individuals implies that this steroid in the dosage employed in the patients studied does not alter the antigenic state of the homograft nor does it affect by absorption from the transplanted skin the immune reactions of the host The lack of significant change in protein fractions and particularly in gammaglobulin correlates with the failure of other investigators to identify specific serum antibodies to homografts [8] Our inability to elicit eosinophilia during homograft rejection similar to that observed by other workers [9, 10] might be due to the failure of the small homografts employed in this experiment to supply an adequate antigenic stimulus for such a response

Absence of the usual external changes indicating homograft rejection in one adrenalectomized patient and the demonstration that replacement of homografted skin had taken place indicates the need for caution in determining homograft survival by sole reference to overt deepithelialization in the homograft The indolent nature of the rejection was related to the patient s inadequate immune mechanisms as intimated by the preexistence

of marked hypogammaglobulinemia. The additive effect of meticorten maintenance therapy and the patient's neoplastic disease on her immune response is difficult to evaluate. Both meticorten and neoplasia are capable of inducing a negative nitrogen balance which might impair antibody synthesis. Neoplasia has not been demonstrated to affect the immune reaction to certain antigenic substances[11] nor has the malnutrition which it causes.[12] However, properdin which figures significantly in immunity has been demonstrated to be reduced in about one half of one group of cancer patients studied.[13]

CONCLUSIONS

1. Survival time of homografts transplanted to adrenalectomized patients maintained on meticorten did not significantly differ from controls in 3 out of 4 adrenalectomized patients.

2. Meticorten maintenance therapy in adrenalectomized patients failed to influence the survival of their skin when transplanted.

3. It was demonstrated that homograft rejection occurred insidiously in one adrenalectomized patient without the occurrence of overt epithelial denudation.

4. It is recommended when experimental homografting is employed, that male and female skin be homografted interchangeably in order that sex chromosomal studies may be utilized to assay the viability of homografts should external *objective evidence of rejection be lacking.*

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CRYOBIOLOGY *

STANLEY W JACOB OWEN E OWEN SAMUEL C COLLINS
AND J ENGLEBERT DUNPHY

Although the term cryobiology may not be found in present day dictionaries studies on the effects of cold applied to living organisms offer exciting possibilities to the medical investigator as a means for long term tissue preservation as a tool in transplantation and cancer research and for potential therapeutic value Our interest ranges from the effects of extreme cold on isolated cells to the use of low temperatures for whole organ preservation

The present study demonstrates the survival of tissues frozen to —272 2°C or within 1° of absolute zero (—273 16°C) There remains however a formidable gap between knowledge needed to maintain viability of frozen cells or thin slices of tissue and our ability to maintain the viability of a frozen organ

It is of course known that a solidly frozen kidney will not resume normal function An entire kidney frozen to low temperatures and thawed by conventional means in addition to functional loss exhibits gross or microscopic evidence of cracking Two techniques to obviate fragmentation of a whole kidney frozen to —180°C will be described

METHOD

Tissues Human conjunctival cells saturated with 6% glycerol human spermatozoa without glycerol human spermatozoa pretreated with 1 part glycerol to 9 parts semen the Coksackie virus without glycerol and dog or rabbit skin previously immersed in 15% glycerol saline were frozen to —272 2°C After this low temperature had been maintained for 30 minutes the material was thawed in a 26°C water bath and subsequently tested for viability

All tissues were enclosed in sealed glass ampules and placed on a specially constructed brass plate under which liquid nitrogen was circulated The ampules were covered by glass wool for insulation and the plate cooled by circulating the liquid nitrogen When the temperature of the ampules reached —180°C over a 2 hour period the glass containers were freed from the plate and placed into a Dewar flask with side arm containing liquid helium (Freeing of the ampules from the plate is facilitated by inserting a layer of grain alcohol between them and the plate prior to freezing) The vapor pressure was lowered to 80 μ Hg equivalent to —272 2°C by applying a vacuum pump to the side arm of the Dewar flask holding the liquid helium After 1/2 hour at this temperature the vacuum was released and the removed ampules thawed in a 26°C water bath

The human conjunctival cells were tested for viability in tissue culture by a method described by Chang[1] Motility was used as the criterion for

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spermato on survival. Viability of the Coksackie virus was determined by culture. Autogenous transplantation of rabbit and dog skin was the standard for studying survival of these tissues.

Organs. The right kidney of a dog was removed with the animal under nembutal anesthesia and the vasculature flushed with dilute heparin saline solution. The kidney was then placed into saline solution maintained at 4 C. After a thermocouple had been deeply inserted into the kidney substance freezing to −180 C. was accomplished by one of the following two techniques:

1. Helium gas which boils at −269 C. was circulated through a copper coil immersed in liquid nitrogen entering the renal artery at −193 C. under 5 lbs pressure through a metal cannula. The gas then emerged via the renal vein. It required 2½ hours for the temperature in the center of the kidney to reach −180 C. The kidney was thawed to 4 C. over a half hour period by helium gas at room temperature passed into the renal artery.

2. In the second technique the kidney was placed on the upper surface of a brass plate 1/4 in. thick and 4 in. in diameter, into which a copper coil had been built. One end of the coil was seated in a small Dewar flask containing liquid nitrogen and the other end attached to an ordinary laboratory vacuum pump. The kidney, enclosed in glass wool insulation, was allowed to freeze from one side to the other. After 2 to 2½ hours when the thermocouple in the center of the kidney read −180 C., thawing was commenced by passing ordinary nitrogen gas through the plate gently warming it and the kidney to 4 C. Either microscopic examination or autogenous transplantation to the neck vessels was then undertaken.

RESULTS

Human conjunctival cells presaturated with 6% glycerol and frozen to within 1° of absolute zero will continue to proliferate in tissue culture after thawing. The growth appeared heavy but quantitative studies were not made.

Ten per cent of human spermatozoa without added glycerol will be motile when thawed from −272.2 C. When 1 part glycerol to 9 semen was added before freezing 60% exhibited motility after thawing.

The Coksackie virus will survive −272.2 C. without prior glycerol saturation.

Autogenous rabbit and dog skin pretreated with glycerol will "take" when transplanted orthotopically. Two out of 2 segments of rabbit skin and 3 out of 3 pieces of dog skin remained viable.

Microscopic sections of entire kidneys frozen by either perfusion or on the plate to −180 C. show only a minimal disruption of the glomerular and tubular pattern similar to the changes which occur when a kidney is maintained outside the body in 4 C. saline for 3 to 4 hours. There was neither gross nor microscopic evidence of fragmentation.

A dog kidney frozen and thawed by the plate or perfusion method will permit the passage of a clear glomerular filtrate for upwards to 2 hours. The filtrate remains grossly clear even when transplantation follows freezing to −180 C. if plate or perfusion thawing is utilized. If, however, a kidney is thawed from −180 C. by immersion in 4 C. saline solution increased glomerular damage as evidenced by a definitely bloody filtrate occurs.

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AND J ENGLEBERT DUNPHY

Although the term cryobiology may not be found in present day dictionaries studies on the effects of cold applied to living organisms offer exciting possibilities to the medical investigator as a means for long term tissue preservation as a tool in transplantation and cancer research and for potential therapeutic value Our interest ranges from the effects of extreme cold on isolated cells to the use of low temperatures for whole organ preservation

The present study demonstrates the survival of tissues frozen to —272 2°C or within 1° of absolute zero (—273 16°C) There remains however a formidable gap between knowledge needed to maintain viability of frozen cells or thin slices of tissue and our ability to maintain the viability of a frozen organ

It is of course known that a solidly frozen kidney will not resume normal function An entire kidney frozen to low temperatures and thawed by conventional means in addition to functional loss exhibits gross or microscopic evidence of cracking Two techniques to obviate fragmentation of a whole kidney frozen to —180°C will be described

METHOD

Tissues Human conjunctival cells saturated with 6% glycerol human spermatozoa without glycerol human spermatozoa pretreated with 1 part glycerol to 9 parts semen the Coksackie virus without glycerol and dog or rabbit skin previously immersed in 15% glycerol saline were frozen to —272 2°C After this low temperature had been maintained for 30 minutes the material was thawed in a 26°C water bath and subsequently tested for viability

All tissues were enclosed in sealed glass ampules and placed on a specially constructed brass plate under which liquid nitrogen was circulated The ampules were covered by glass wool for insulation and the plate cooled by circulating the liquid nitrogen When the temperature of the ampules reached —180°C over a 2 hour period the glass containers were freed from the plate and placed into a Dewar flask with side arm containing liquid helium (Freeing of the ampules from the plate is facilitated by inserting a layer of grain alcohol between them and the plate prior to freezing) The vapor pressure was lowered to 80 μ Hg equivalent to —272 2°C by applying a vacuum pump to the side arm of the Dewar flask holding the liquid helium After ½ hour at this temperature the vacuum was released and the removed ampules thawed in a 26°C water bath

The human conjunctival cells were tested for viability in tissue culture by a method described by Chang [1] Motility was used as the criterion for

* From the Department of Surgery Harvard Medical School The Fifth Surgical Service and Sears Surgical Laboratory Boston City Hospital The Cryogenic Engineering Laboratory Massachusetts Institute of Technology Supported in part by a Research Grant C 3930 from the National Cancer Institute Public Health Service and aided by a grant from the Greater Boston Chapter of the Massachusetts Heart Association

spermatozoa survival. Viability of the Coksackie virus was determined by culture. Autogenous transplantation of rabbit and dog skin was the standard for studying survival of these tissues.

Organs. The right kidney of a dog was removed with the animal under nembutal anesthesia and the vasculature flushed with dilute heparin saline solution. The kidney was then placed into saline solution maintained at 4°C. After a thermocouple had been deeply inserted into the kidney substance, freezing to —180°C. was accomplished by one of the following two techniques:

1. Helium gas which boils at —269°C. was circulated through a copper coil immersed in liquid nitrogen entering the renal artery at —190°C. under 3 lbs. pressure through a metal cannula. The gas then emerged via the renal vein. It required 2½ hours for the temperature in the center of the kidney to reach —180°C. The kidney was thawed to 1°C. over a half hour period by helium gas at room temperature passed into the renal artery.

2. In the second technique, the kidney was placed on the upper surface of a brass plate 1¼ in. thick and 4 in. in diameter, into which a copper coil had been built. One end of the coil was seated in a small Dewar flask containing liquid nitrogen and the other end attached to an ordinary laboratory vacuum pump. The kidney enclosed in glass wool insulation was allowed to freeze from one side to the other. After 2 to 2½ hours, when the thermocouple in the center of the kidney read —180°C., thawing was commenced by passing ordinary nitrogen gas through the plate gently warming it and the kidney to 4°C. Either microscopic examination or autogenous transplantation to the neck vessels was then undertaken.

RESULTS

Human conjunctival cells presaturated with 6% glycerol and frozen to within 1° of absolute zero will continue to proliferate in tissue culture after thawing. The growth appeared heavy but quantitative studies were not made.

Ten per cent of human spermatozoa without added glycerol will be motile when thawed from —272.2°C. When 1 part glycerol to 9 semen was added before freezing, 60% exhibited motility after thawing.

The Coksackie virus will survive —272.2°C. without prior glycerol saturation.

Autogenous rabbit and dog skin pretreated with glycerol will "take" when transplanted orthotopically. Two out of 2 segments of rabbit skin and 3 out of 3 pieces of dog skin remained viable.

Microscopic sections of entire kidneys frozen by either perfusion or on the plate to —180°C. show only a minimal disruption of the glomerular and tubular pattern similar to the changes which occur when a kidney is maintained outside the body in 4°C. saline for 3 to 4 hours. There was neither gross nor microscopic evidence of fragmentation.

A dog kidney frozen and thawed by the plate or perfusion method will permit the passage of a clear glomerular filtrate for upwards to 2 hours. The filtrate remains grossly clear even when transplantation follows freezing to —180°C. if plate or perfusion thawing is utilized. If, however, a kidney is thawed from —180°C. by immersion in 1°C. saline solution increased glomerular damage as evidenced by a definitely bloody filtrate occurs.

DISCUSSION

If we consider that the deterioration of frozen tissues obeys the Arrhenius equation for rate processes it is difficult to conceive that temperatures below —79° are necessary for long term storage Yet Parkes demonstrated that fowl spermatozoa pretreated with glycerol lost motility after several weeks at —79°C and after 1 year at —196°C We are, at the present, quantitating long term survival of human conjunctival cells saturated with 6% glycerol stored at – 79°C and —196°C No one can say whether or not temperatures approaching the absolute zero will be necessary for indefinite preservation of mammalian tissues but that mammalian tissues will withstand these temperatures is of interest

As previously mentioned a solidly frozen dog kidney will not regain normal function following transplantation [2] The irreversible damage occurs between —2 to 4°C A kidney frozen by usual techniques to —1°C and retransplanted will exhibit marked glomerular dysfunction producing a bloody glomerular filtrate The techniques for freezing and thawing here described do not enable a solidly frozen kidney to function normally after transplantation but they do minimize glomerular damage as evidenced not only by the microscopic findings but by the production of clear glomerular filtrate even in a kidney frozen to —180°C, thawed, and retransplanted

When an entire organ is frozen by a method which permits a shell of ice to form on the surface, the continuous expansion of water during the freezing process causes fragmentation Both the perfusion and plate techniques, by eliminating the encircling shell of ice, enable the water to expand without fracturing the organ Plate or perfusion thawing is more rapid than allowing the kidney to thaw at room temperature The fact that plate or perfusion thawing of a kidney causes less glomerular derangement than occurs when an entire organ frozen to —180°C is thawed in 4°C saline remains unexplained

CONCLUSIONS

1 Human conjunctival cells will maintain viability when saturated with 6% glycerol frozen to —272 2°C and thawed in a 26°C water bath

2 Sixty per cent of human spermatozoa saturated with glycerol subjected to temperatures approaching the absolute zero are motile after thawing Ten per cent of human spermatozoa without glycerol remain motile after ½ hour at —272 2°C and thawing

3 Dog and rabbit skin saturated with glycerol can be frozen to —272 2°C, thawed and autologously transplanted with a "take"

4 Techniques to minimize the physical effects of freezing to very low temperatures on a dog kidney are presented

We are indebted to Dr Robert S Chang Harvard School of Public Health, for determining viability of the human conjunctival cells following freezing

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BACTERIAL STUDIES OF THE BURN WOUND IN RELATION TO THERAPY AND SKIN GRAFTING PROCEDURES*

GILBERT G. LADE, JOSEPH A. BASS, STEPHEN R. LEWIS
AND TRUMAN G. BLOCKER, JR.

This study is an effort to define more closely the effect of therapy on the bacteria found in open wounds. Differential and total bacterial counts and the take of skin grafts on a large number of granulating burn wounds were obtained. These provided objective data for comparing different forms of topical and systemic antibacterial therapy.

METHOD

The techniques for obtaining the bacterial counts have been described previously; they consisted of a closed wound washing chamber from which dilution cultures for counting could be obtained and a direct stamping grid that could be transferred directly to a culture plate. Cultures were taken only after the surface had been washed with pHisoHex and rinsed with water to remove exudates and debris. This left the residual organisms that were covered by skin grafts. It was assumed that those remaining represented the same percentage of types as total present before preparation if a standard method of preparation was used. All preparation, culturing and grafting were done by the same persons to provide uniformity. The assignment of treatment schedules was done in a random manner by an individual not related to the study itself. The divisions of the study and the results are described.

RESULTS

Effect of Topical Therapy. Bacterial counts were made from 18 areas treated 24 hours before grafting with wet compresses and compared with counts from 17 areas covered only by dry dressings prior to skin grafting. Table 1 shows the results. The counts in the dry areas averaged twice those found in the area treated with soaks, yet graft takes were the same in both groups.

Table 1. Effect of Therapy on Bacterial Count and Graft Takes

THERAPY	AVERAGE COUNTS (1)	AVERAGE GRAFT TAKE (2)
Dry Dressings	2636	83%
Wet Dressings	1160	81%
Systemic Antibiotics	2824	83%
No Systemic Antibiotics	2630	74%

(1) Average counts in number of bacteria per square inch.
(2) Average take compared amount applied and that present and viable at the first and second postoperative dressing changes using the palm of the hand as a 25 square inch reference.

* From the Department of Surgery (Plastic and Maxillofacial), University of Texas Medical Branch, Galveston. Supported by a grant from the U. S. Army, Contract DA-49-007-447.

Differential cultures were obtained from open wounds on more than 100 patients on whom local saline and glycerine dressings were compared with dressings containing various antibiotics These findings are listed in Figure 1 No significant bacteriological differences were noted between those treated with saline glycerine, or topical antibiotics The percentages of skin graft takes were also comparable

These findings suggest that pregrafting soaks reduce the number of bacteria present but do not enhance skin graft takes and that topical antibiotics neither affect the bacteria present nor the take of skin grafts

Effect of Systemic Therapy. Twenty seven patients received systemic antibiotics for 3 days before and 3 days after skin grafting and were compared with 24 patients who received none The bacterial counts and graft takes are shown in Table 1 The counts were almost the same, but the skin graft takes were somewhat better in the antibiotic treated group

The same differential cultures as mentioned under topical therapy were regrouped according to patients receiving systemic antibiotics and those receiving none The findings are compared in Figure 2 Antibiotics appeared to reduce the percentage of susceptible bacteria present while nonsensitive species such as pseudomonas were increased Skin graft takes were better when antibiotics which reduced known pathogens were used

Studies on systemic therapy suggest that antibiotics do not reduce the total number of bacteria present but do reduce sensitive species If the bacteria inhibited are harmful to skin grafts graft take is improved At the same time however, nonsensitive species are increased because they utilize the nutrient available as the sensitive organisms are eliminated, thus keeping the total count about the same

Effect of Skin Grafting Table 2 compares bacterial counts and graft take in 58 cases The counts had no consistent relationship to graft take

Differential counts were taken from 14 areas before and after skin grafting on which graft takes were less than 50% and the loss attributed to infection These were compared with cases having a graft take greater than 90% The results are shown in Table 3 and it can be seen that the relative incidence of most common bacteria found were very close in the two groups

These results with skin grafts suggest that bacteria themselves may not be

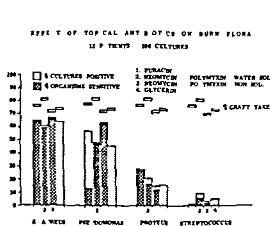


Fig 1

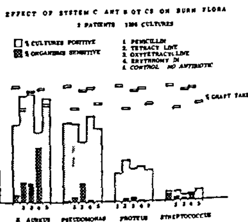


Fig 2

Table 2 Effect of Bacterial Counts on Graft Takes

TAKE	AVERAGE COUNT (1)	CASES
[illegible] than 90	32[illegible]2	33
7[illegible] 90%	5150	7
[illegible]	2700	16
less than 50%	5000	2

(1) Bacteria per square inch

Table 3 Bacteria and Graft Failure

ORGANISM	POOR GRAFT TAKE (1)	EXCELLENT GRAFT TAKE (2)
S aureus	6[illegible]% †	68%
Pseudomonas	66%	63%
Proteus	26%	27%
Hemolytic Strep	6%	7%

† Percentage of total cultures positive for a given organism
(1) Poor is less than a 50% take
(2) Excellent is a take 90% or greater

harmful to graft take if the areas are mechanically cleansed and that graft loss may be due to other factors that permit bacterial overgrowth inasmuch as there was no appreciable difference in organism types between areas having excellent and poor takes

Fate of Bacteria Covered with Skin Grafts Skin grafts on 38 areas were peeled back at either 1, 2 4 8 12 or 24 hours postoperatively and quantitative counts taken The difference in these counts and those present before grafting are compared in Table 4 and indicate the bacteria decreased rapidly once skin coverage was obtained

The findings were the same when nonviable homografts were used which implies that this reduction is due to properties within the granulation tissue rather than anything antibacterial in the skin grafts

Table 4 Reduction of Bacteria after Grafting

PRE GRAFT AVERAGE COUNTS	TIME	POST GRAFT AVERAGE COUNT	PERCENTAGE OF BACTERIA REMOVED
310	1 hr	316	0%
29[illegible]	2 hr	85	71%
21[illegible]2	4 hr	414	80%
993	8 hr	3[illegible]	97%
2607	24 hr	110	96%

EFFECT OF TOTAL BODY IRRADIATION ON BIOCHEMICAL SEQUENCES IN GRANULATION TISSUE *

DALE B FLICKINGER, KEITH D J VOWLES AND J ENGLEBERT DUNPH

The effect of sublethal total body irradiation on the various compone and mechanisms of wound repair has not been extensively studied althou considerable research has been done on other biological aspects of total b irradiation The present study was undertaken to appraise the effects sublethal total body irradiation on the production of granulation tissue collagen concentration in the primarily closed wound

METHOD

This experiment was conducted using young growing, albino male weighing initially between 150 and 200 gm The animals were fed a stand diet

Under light ether anesthesia all animals were weighed and blood samp taken Transverse incisions were made through the skin and pannicu carnosis on the anterior and posterior regions of the back Two iva sponges were implanted in the subcutaneous tissue of each incision by technique previously described by this laboratory,[1] and the incisions w sutured primarily The ivalon sponges were harvested at 4 day intervals to 16 days and the new granulation tissue they contained was analyzed dry weight for hydroxyproline using the method of Neuman and Loga Collagen concentration was calculated by multiplying the hydroxyprol concentration by the factor of 7 46 of Neuman and Logan All anim were weighed at 4 day intervals No antibiotics or other drugs were ministered

The animals were placed into three groups as follows *Group I* Sixty t animals served as controls They were treated as described above Blood cou were done *Group II* Immediately after wounding, 48 animals were dividually given 200 r total body irradiation using a TH 2 filter (1 0 25 Cu , 0 4 Sn) 50 cm distance, 15 ma 250 K V P for 5'33" Blood cou were done prior to irradiation and at intervals after irradiation Spo biopsies of wounds were taken as described *Group III* Immediately af wounding 42 animals were given 600 r total body irradiation which is ab the LD 50[3] within 30 days A TH 2 filter was used at 50 cm distance, 15 m 250 K V P for 16'40" Blood counts and sponge biopsies of the granulati tissue were taken as in Group II

RESULTS

Group I There was a steady gain of weight and the increase in collag in the wound conformed to the normal pattern previously described in t laboratory (Fig 1, 2, 3)

Group II Slight depression of collagen formation was noted at 8 and days in comparison with the control animals but at the sixteenth day, the was no significant difference between control and irradiated groups

* From the Department of Surgery Harvard Medical School Fifth Surgical Service Sears Surgical Laboratory Boston City Hospital Supported by a research grant RG 5919(from the Division of Research Grants Public Health Service

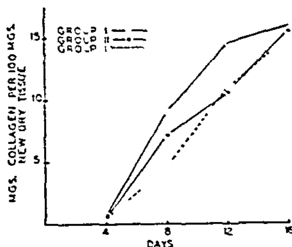


Fig 1 Comparative curves of collagen concentration in closed wounds of control and irradiated animals.

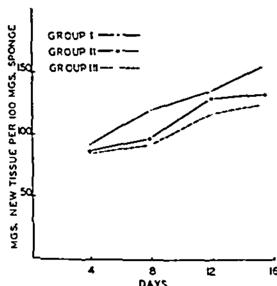


Fig 2 Comparative curves of new tissue formation (dry weight) in closed wounds of control and irradiated animals

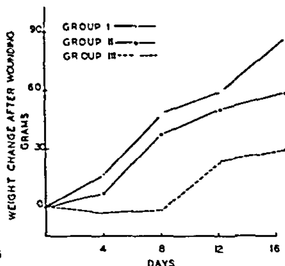


Fig 3 Comparative curves of weight gain following wounding in control and irradiated animals

animals in the irradiated group gained weight but were generally below the weights of the control animals A mild leucopenia was noted in 24 hours after irradiation but white blood counts done on the fourth day had returned to prewounding levels

Group III There was anemia leucopenia, and marked suppression of weight gain in all animals Forty three per cent of the group were dead within 30 days Collagen production was moderately depressed on the eighth and twelfth days but was within normal limits by the sixteenth day after wounding The white blood cell count was lowest on the fourth day determinations but was rising by the twelfth day Red blood cell counts decreased steadily throughout the sixteen day period There was slight weight loss present until the twelfth day determinations when a gain was noted

DISCUSSION

Among the prominent effects of total body irradiation are those associated with suppression of hematopoiesis, and ulcerative destruction of the

gastrointestinal tract mucosa A direct effect of sublethal total body irradiation on connective tissue is not established Abnormality in the granulation tissue of a healing wound could be a reflection of anemia leucopenia or hypoproteinemia in radiation sickness or of direct local tissue damage Hematologic and gastrointestinal symptoms may become evident promptly but a latent period of symptoms and anemia is common in sublethal radiation

It has been established from the experimental data that collagen concentration in granulation tissue obtained by the sponge biopsy technique from a primarily closed wound is normal by 16 days in the totally irradiated rat even with concurrent anemia leucopenia and weight loss Radakovich[4] noted no retardation in the closure (contraction) of open wounds in rats subjected to total body irradiation varying up to 650 r Raventos[5] reported that in rats receiving 500 r total body irradiation there was no difference by 14 days in the strength of primarily closed abdominal incisions between control animals and irradiated animals

After studying biological effects of the atomic bomb Oughterson and Warren[6] reported prolonged wound healing among the Japanese victims and coincidently with the appearance of symptoms of radiation illness granulation tissue became pale and edematous Pearse and Payne[7] stressed the importance of early definitive treatment of such wounds If wound healing had not progressed within the first 2 weeks prior to onset of radiation symptoms even the casualty receiving a sublethal dose of ionizing radiation was faced with wound breakdown infection and perhaps death from a relatively minor injury Russian investigators[8] have reported that in irradiated experimental animals operative procedures done during the climax of radiation effect were accompanied by a higher mortality rate than similar operations done immediately after radiation exposure

It has been military medical practice not to close war wounds until a long enough period of time has passed to ascertain the extent of tissue devitalization and to perform delayed secondary wound closures On the basis of present evidence it would appear that early closure of wounds in persons who have sustained total body irradiation is desirable and that normal healing may be expected

SUMMARY

Experimental wounds closed immediately after sublethal total body irradiation have a pattern of connective tissue proliferation within the normal range

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CANNULATION OF THE LYMPHATICS OF THE LOWER EXTREMITY *

JAMES R. SMITH, L. FRANK DUNTON, JACOB M. PROTAS,
TRUMAN G. BLOCKER, JR., ROBERT M. COOLEY AND STEPHEN R. LEWIS

The technique for cannulation of the lymphatics of the lower extremity has been utilized on clinical subjects and experimental animals to study the anatomy and hydrodynamics in normal and abnormal states.

METHOD

Direct Sky Blue dye was injected subcutaneously (0.1 to 0.2 ml. of 4%) in the web between the first and second toes in patients and dogs. This dye was rapidly picked up by the lymphatics of the lower extremity. After 5 to 10 minutes an incision was made across the dorsum of the foot 2 to 3 inches above the metatarsophalangeal articulation. The lymphatic trunks colored by the blue dye were readily visualized and a 27 gauge needle clipped near the shaft and inserted in a polyethylene cannula was introduced. A 4-0 silk suture was used to secure the needle into the lymphatic; the polyethylene tubing was either sutured or taped to the foot to insure immobility. Cannulas remained patent over a long period of time because the lymph fluid did not clot. One patient's lymphatics with the inserted cannulas remained open 6 weeks. Daily injections of normal saline were given to ascertain patency.

An electromonometer bridge with amplifier connected to an electrocardiograph was used to measure intralymphatic pressure. These pressure readings were taken immediately after cannulation. It was common practice to cannulate a vein on the lateral aspect of the foot so that both lymphatic and antegrade venous pressures could be taken. Retrograde lymphatic and venous pressures were measured in the inguinal region. Arterial and subcutaneous pressures were taken at various sites.

Thirty-two patients and 30 dogs were studied. The procedure was usually done bilaterally. Due to technical difficulty 2 of the patients could not be cannulated. The legs studied included normal legs, those with lymphedema, hemangiomas, posttraumatic ulcers, varicose veins and malignant lesions.

After pressure readings were taken, lymphangiograms were made. Fifty per cent miokon was used as the contrast material. The procedure was usually done under local anesthesia so that the patient was able to trace the injection flow of the medium from the painful sensations that occurred. The patient thereby determined for us the amount of medium necessary to reach the inguinal region. This was extremely helpful, as the amount varied from patient to patient. The same sensation was noted with injections of saline and RISA. The table was placed in a Trendelburg position while the miokon was injected with a tuberculin syringe. A wedge filter was used to x-ray the lower extremity. The most revealing pictures proved to be those taken 5 minutes after the initial introduction of the miokon. Subsequent films were

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* From the Department of Surgery (Plastic and Maxillofacial), University of Texas Medical Branch, Galveston. Supported by the Rachel and Ben Vaughan Foundation, Corpus Christi, Texas.

taken to follow the dispersion of the medium This same medium was injected into the cannulated veins and venograms taken concomitantly with the lymphangiograms

Radioactive material (RISA) was used to measure lymphatic flow time This was done on patients during radical neck dissections near the end of the operative procedure RISA (1 ml ~ 10 μc/ml) was injected into the lymphatics of the foot and samples of lymph fluid were collected simultaneously from the thoracic duct and a systemic vein nearby at specific time intervals Radioactivity was determined by a well scintillation counter RISA was considered the best radioactive material to use intralymphatically since the lymphatics selectively picked it up when injected subcutaneously Other materials such as radioactive sodium are picked up primarily by the blood capillary system [1] Comparable flow studies were made on dogs

RESULTS

The small caliber of the human lymphatic trunks is a major factor responsible for the technical difficulties encountered Their cannulation was not a problem in dogs because the lymphatic trunks are much larger Stabilization of the catheter was another problem factor which became complicated when the patient was moved These technical difficulties decrease proportionally with the experience of the operating team

The discoloration on the dorsum of the foot created by the Sky Blue dye usually disappeared within 2 months in normal extremities In the pathological extremities (lymphedema and varicosities) the discoloration disappeared in a much shorter time An intradermal injection of this dye into the neck region resulted in a persistence of the discoloration for approximately 12 months This type of injection should be avoided

The lymphatics of the hand have been identified by a method similar to that used in the feet

No mortalities resulted from these studies the morbidity has been directly correlated to the amount of miokon injected Three patients who received in excess of 20 ml of miokon dye in a single extremity exhibited moderate degrees of cellulitis and lymphangitis These patients were given antibiotics and absolute bed rest The majority of our patients were allowed to ambulate following lymphangiography 12 were done as out patients

A general classification similar to that discussed by Kinmonth [2] was made on the lymphedema patients Representative x rays of the ectatic hypoplastic and normal lymphatic vessels were noted in this group

The thoracic duct was visualized in the dog radiographically subsequent to miokon injection through the leg lymphatics A photograph of a patient with a hemangioma of the leg is shown in Figure 1 X ray photographs of the lymphatics of the leg thigh inguinal and iliac region are shown in Figures 2 3 4 A concomitant lymphangiogram and venogram is shown in Figure 5 Intralymphatic pressures flow studies and readings as recorded in all of our clinical and laboratory material will be reported in detail in subsequent publications

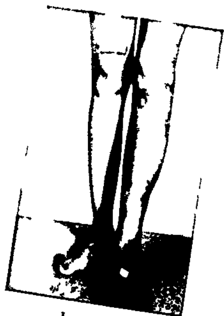


Fig 1



Fig 2



Fig 3



Fig 4



Fig 5

SUMMARY

Cannulation of the lymphatics of the lower extremity may be used as a tool to study the anatomy and physiology of this extremity The normal and the pathologic extremity may be studied

Successful cannulation of the lymphatics of the lower extremity has been utilized clinically and experimentally in trauma, malignant diseases and lymphedema

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HEALING OF TENDONS *

Immobilization Versus Active Motion

JAMES H HENDRIX, JR AND HEBER C ETHRIDGE

The objectives of the study were to compare the results of immobilization and early active motion in free tendon grafts of the hand

Many studies of the physiologic and healing processes of tendons have been reported in the last fifty years To our knowledge none of this work has been done on material comparable to that of the human hand but rather on more gross structures such as the tendons of dogs

METHOD

The Cynomologous monkey was used in this study since its hand is very similar anatomically to the human hand In all, the sublimis tendon was discarded In 1 group of animals the profundus tendon was removed completely then reinserted as the graft In another group the palmaris longus tendon with paratenon was used as the graft Some animals of each group were immobilized in plaster for 3 weeks while others had pressure dressings for from 1 to 7 days followed by active motion Exploration of these operative sites was carried out at 3 week and 3 month intervals

RESULTS

Findings were as follows 1) marked scarcity of adhesions at the anastomotic sites in the palm and at the distal anastomotic site 2) a large amount of adhesions along the graft in the finger, 3) fair to good function of the grafted finger in spite of the presence of adhesions 4) profunda and/or sublimis grafts functioned better than grafts of the palmaris longus with paratenon, and with less contracture, 5) a significant difference in the results in the case of immobilization versus active motion could not be determined If good primary healing was obtained without infection or hematoma formation, the resulting function was about the same 6) Inability of the animal to cooperate and difficulty in postoperative occupational therapy and physio therapy compromised the results

* From the University of Mississippi Jackson

CONCLUSIONS

1. This study is presented as a preliminary report, as only 12 animals could be evaluated.

2. The use of the sublimis tendon as a graft whenever possible may be worth greater consideration.

3. Seven to 11 days immobilization to obtain healing of the skin, followed by mild active and passive motion, may be the treatment of choice.

4. Evaluation of results in human subjects by an experienced surgeon using different techniques and times of immobilization will continue to produce the most accurate and useful information.

BIOLOGICAL REACTIONS TO COLLAGEN TRANSPLANTS *

ERLE E. PEACOCK, JR AND JERRY M PETTY

Successful restoration of animal and human tendon defects by grafting homologous and heterologous tendons has been frequently reported in medical literature.[1] Review of these reports raises the question of whether the original fibroblasts and collagen persisted or whether transplanted cells were replaced by host cells which ultimately replaced the fibers as well Transfer of tendons previously fixed in alcohol or formalin suggests that the role of fibroblasts is unimportant, but the majority of these experiments were performed upon the Achilles tendon of animals, a tendon with such a small amplitude of motion that, even after replacement by disorganized scar tissue, it functions satisfactorily In spite of the number of recorded tendon transfer experiments, there is little evidence concerning the fate of cells and fibers. Replacement of cells seems more likely than replacement of fibers, since fiber replacement would require a powerful and specific collagenase which has not been identified in human beings or laboratory animals

Because clinicians have long recognized the superiority and availability of autogenous tendon grafts for the repair of human tendon injuries, there has not been a pressing need to answer these questions Recently, however, we have found that restoration of selected flexor tendon injuries by means of a composite tissue homograft of the entire flexor mechanism may offer advantages over conventional single tendon autografts[2] Moreover, all homografts, including skin, kidney, blood vessels, and endocrine organs, include the transfer of homologous collagen and ground substance As part of the general investigation of the homograft rejection phenomenon it may be worth while to study the antigenicity and host reaction to isolated fractions of complex tissue grafts In an attempt to learn the fate of composite tissue tendon homografts used in the restoration of human flexor tendons and to evaluate the

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importance of collagen in the rejection of other tissue homografts we have performed the following experiments

METHOD

Flexor tendons from the forearm of adult mongrel dogs were transplanted into the subcutaneous tissue of the abdominal wall of rabbits The tendons were sutured to the external oblique fascia to maintain longitudinal tension Three types of tendon preparations were implanted 1) fresh tendons washed in saline and transplanted immediately 2) tendons stored at 37°F in buffered saline and antibiotics for 1 2 4 and 6 weeks and 3) tendons stored for 4 weeks at 37°F followed by digestion with 3 fresh applications of trypsin to clear the grafts of cells and noncollagenous debris Biopsies were taken at 48 hour intervals for the first 6 weeks and at weekly intervals during the next 6 months

A second set of experiments was designed to evaluate the antigenicity of homologous and heterologous tendon grafts The tail tendons from 20 C_3H mice were transplanted subcutaneously into the abdominal wall of 20 C mice Ten tendons were transplanted immediately after having been washed in cold saline to remove blood and tissue fragments An additional 5 tendons were treated with 3 applications of trypsin and another group of 5 tendons was fixed in formalin before transplantation If any group contained significant antigens their location and stability might be determined by comparing the antibody response Five weeks later the mice receiving the tendons were challenged by a skin graft from the same C_3H mouse which had donated the original tendon Eight pairs of C_3H and C mice served as controls to measure the time interval for first and second set rejections in this experiment

In a third experiment fresh tendon grafts or neutral salt reconstituted and lyophilized collagen prepared from an acetic acid extraction of dog tendons were implanted in the subcutaneous tissue of 4 rabbits Four weeks to 6 months later the rabbits were challenged with a skin graft from the same dog which had donated the tendon grafts and reconstituted collagen At the same time the rabbits sera were tested for dog collagen antibodies by complement fixation tests Reconstituted lyophilized collagen from the original acetic acid extraction was the antigen and a sensitized sheep red cell hemolysis system served as an indicator In addition to the usual titrations of complement and amboceptor serial dilutions of both antigen and serum were tested

RESULTS

Implantation Experiments Biopsies from tendon heterografts on the second and fourth day after transfer revealed normal collagen and cells The collagen bundles were slightly separated but the staining qualities of both fibers and fibroblasts were unchanged By the sixth day the fibroblasts showed definite pyknosis which by the eighth day had progressed to frank karyorrhexis When the tendons were subjected to lateral stress a reduction in transverse cohesiveness was detected The next 6 days showed a thorough removal of cells and noncollagenous debris and by the 16th day there was an absence of basophilic staining and only collagen remained While fibroblasts were being removed the tendon became surrounded by mononuclear host cells Grossly the graft was encapsulated by a soft gray gel This gel on

microscopic examination, consisted of lymphocytes and macrophages imbedded in a loose fibrin clot Eosinophils were rare and polymorphonuclear granular leukocytes were seldom seen following aseptic transplants Infected grafts soon became invaded by white blood cells, but aseptically transferred tendons did not stimulate a granulocyte response.

After the graft had been cleared of its original cells, invasion by host cells began At this time the graft appeared exactly like the tendons treated with trypsin During the sixteenth through the thirtieth day a gradual invasion of the collagen by host cells continued until the transplant was quite cellular again The surrounding capsule became less cellular and changed from a soft gray gel into a fibrous sheath The sheath did not choke or constrict the graft but was easily separated from the clearly identifiable tendon With the invasion of new cells, the interstices between fibers filled with an amorphous substance that appeared to congeal the collagen bundles into a compact unit. By the fortieth day the initial profuse cellular invasion had somewhat diminished restoring a normal ratio of cells to collagen Six months after transplantation the new cells were still scattered in a disorganized fashion throughout the graft A few cells appeared to develop polarity, but the majority remained round or cuboidal, never resembling the mature elongated fibroblasts of adult tendon (See Fig 1 and 2)

The same sequence of cell death, clearing, and invasion by mononuclear host cells occurred whether the graft was transferred immediately or stored for 6 weeks The fate of the cells and ground substance was the same for all grafts and, as far as could be determined by these experiments, the collagen and longitudinal strength of the graft was not affected by refrigeration The behavior of trypsin digested grafts differed from untreated grafts only in the time interval between implantation and invasion Having already been cleared of cellular elements by *in vitro* digestion, the host cells invaded the trypsin cleared collagen with no delay Six weeks after transfer there was no difference in the appearance of trypsin treated grafts and untreated tendons

Antigenicity Experiments. In the control group, full thickness skin grafts



Fig 1 Left Normal dog tendon at time of transfer to rabbit Right Dog tendon 11 days after transfer to rabbit Note complete absence of cells



Fig 2 Left Dog tendon 17 days after trans-

A STUDY OF THE RENAL EXCRETION OF CALCIUM AND PHOSPHORUS IN PATIENTS WITH RENAL CALCULI *

SHERIDAN W SHIRLEY, KARLMAN WASSERMAN, EDGAR BURNS, AND RICHARD E PADRNOS

The etiology of renal calculous disease is incompletely understood It is known that the common constituents of renal stones are calcium, phosphorus, oxalate, magnesium and ammonium, of which calcium and phosphorus occur in the majority This study was designed to evaluate calcium and phosphorus excretion by the kidney in patients with and without renal calculi Specifically, attention was focused on the renal clearance of calcium and phosphorus in relation to the urea clearance An attempt was made to determine if there were quantitive differences in the excretion and reabsorption of calcium and phosphorus in patients with renal calculous disease

METHOD

A total of 42 patients from Charity Hospital, New Orleans, was used in these studies Nine control studies were performed on patients with conditions other than renal disease Twenty seven patients with renal calculous disease and 5 chronically bedridden patients without renal calculous disease were studied All patients had received a regular hospital diet for at least 5 days

After overnight fasting, 2 hour urine specimens were collected and blood samples were drawn Calcium, phosphorus and urea nitrogen determinations were performed on urine and blood [1, 2] Clearance values of calcium, phosphorus and urea were derived by appropriate calculations

RESULTS

These studies demonstrated that the phosphorus to urea clearance ratios were 2 to 3 times greater in patients with renal calculi than in patients

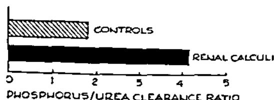


Fig 1 Diagram showing phosphorus to urea clearance ratios in 9 control patients and in 27 patients with renal calculi

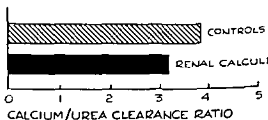


Fig 2 Diagram showing calcium to urea clearance ratios in 9 control patients and in 27 patients with renal calculi

* From the Departments of Urology and Physiology Tulane University School of Medicine and the Tulane Urologic Service Charity Hospital New Orleans Supported in part by a grant from the Mina Haller Research Fund

with normal kidneys, whereas the calcium to urea clearance ratios remained approximately the same. Statistical analyses indicated that phosphorus excretions were significantly elevated in people with renal stones. Calcium determinations revealed slight change from controls The increased clearance of phosphorus was directly responsible for the elevated phosphorus to urea clearance ratio in the patients with renal calculi, as the urea clearance remained approximately the same.

Preliminary data suggested that patients who are chronically bedridden have a phosphorus to urea clearance ratio greater than the controls but less than that of the patients forming renal calculi. Urinary excretion of calcium appeared decreased in patients who have been bedridden for periods of time longer than 100 days.

DISCUSSION

The relationship of disturbed calcium and phosphorus metabolism to renal calculous disease associated with hyperparathyroidism is well known. These patients usually demonstrate persistent hypercalcemia, hypophosphatemia and hypercalcinuria. An increased phosphorus to urea clearance ratio is also observed in such patients

The importance of altered calcium and phosphorus metabolism in renal calculous disease not associated with hyperparathyroidism has not been completely defined. This study confirms the work of previous investigators regarding the calcium metabolism in such patients No significant alteration in serum or urinary calcium was found.

Significant increase of the phosphorus to urea clearance ratios was observed in the group of patients with renal calculous disease. The increased clearance of phosphorus was responsible for the elevation of the phosphorus to urea clearance ratio, as the clearance of urea was not significantly altered.

Whether this effect was mediated by parathormone cannot be determined from this study However, there is experimental evidence[3, 4, 5, 6] that

Table 1. A Study of the Renal Excretion of Calcium and Phosphorus in Patients with Renal Calculi

	MEAN RESULTS												
		PLASMA, MG%			URINE MG%			CLEARANCE, CC. MIN					
PATIENTS	URINE CC/MIN	U	CA	P	U	CA	P	U	CA	P	CA/U	P/U	
1 Controls	62	11 12	9 9	3 4	645 2	17 0	36 9	33 58	1 01	6 33	038	187	
2 Renal Calculi	87	12 9	9 9	3 1	629 3	13 9	67 0	36 3	1.06	14 93	031	428	
3 Patients Chronically Bedridden	44	12 6	9 8	3 4	867 2	13 9	84 0	35 1	71	10 93	017	.321	
4 Variations of Ca & P Diet	51	7 4	9 8	3 5	362 3	21 7	33.5	24 9	1 13	4 88	015	.197	LOW
	76	8 9	9 5	3.2	488 3	16 2	53.5	41 7	1 30	12 71	.031	305	HIGH

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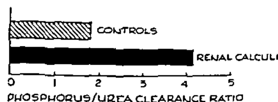


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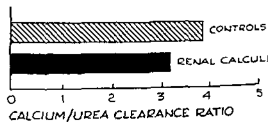


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PATIENTS	U FLOW CC/MIN	U	CA	P	U	CA	P	U	CA	P	CA/U	P/U
1 Controls	62	11 12	9 9	3 4	615 2	17 0	36 9	33.58	1 01	6 33	038	187
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parathormone has a direct action on bone reabsorption, in addition effect on tubular reabsorption of phosphate It is suggested from this *that a hypersecretion of the parathormone affecting only the kidney m* responsible for calculous formation

CONCLUSION

In a series of 27 patients with renal calculous disease it was found th phosphorus/urea clearance was significantly increased There was n tectible alteration in calcium metabolism

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THE RENAL ARTERY HOMOGRAFT *

An Experimental Study

GUY W LEADBETTER JR

Goldblatt s [1] classic experiment demonstrated a definite relationship betw renal ischemia and hypertension Soon afterward Butler [2] associated lateral pyelonephritis and hypertension and reported a dramatic cure eff by nephrectomy The pathology underlying renal hypertension may be renchymal or vascular Parenchymal disease such as glomerular neph chronic pyelonephritis constrictive nephritis etc may be either unila *or bilateral It is obvious a cure is not possible in the presence of bila* parenchymal disease unless an identical twin is available However rem of a unilateral diseased kidney does effect a cure in selected cases [3]

Renal artery disease consisting of atherosclerotic placques art thromboses or emboli congenital strictures of the main renal artery arte and renal arterial aneurysms may all cause hypertension In the nephrectomy has been the treatment of choice in unilateral disease *refinements in arterial surgery however* it is now possible to aggressi approach and successfully cure many types of arterial disease Renal art reparative surgery rather than nephrectomy offers a method for prese tion of renal tissue in unilateral renal disease and is the sole therapy avail for bilateral arterial disease or when a solitary kidney *is affected*

* From the Massachusetts General Hospital Department of Urology Boston

The success of renal artery replacement depends on: 1) as short a period of renal ischemia as possible; 2) adequate postoperative blood flow; and 3) prevention of postoperative stricture and/or thrombosis. In an effort to develop a technique which minimizes these problems and produces the most physiologic results in renal artery replacements, five techniques were used experimentally in dogs, to reconstitute the renal artery and aorta. All grafts were homografts, frozen and sterilized by high voltage x-ray. The techniques were: 1) a standard end-to-side graft (Fig. 1); 2) a spatulated end-to-side graft with (a) the distal lumen facing caudad (Fig. 1 and 3), (b) the distal lumen facing cephalad (Fig. 3); 3) an aortic segment containing both renal arteries as described by Poutasse and Humphries;[4] 4) a "patch" graft obtained by excising the renal artery with a 3 to 4 mm. margin of aortic wall (Fig. 2).

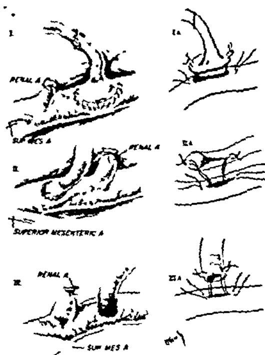


Fig 1—I and IA Technique for applying the "patch" graft, II and IIa Technique for applying the spatulated end to side graft facing caudad III and IIIa Technique for applying the end to side graft



Fig 2 Technique for obtaining the "patch" graft Note the natural funnel



Fig 3 Grafts in series along the aorta for blood flow measurement A) Spatulated end to side graft facing caudad B) Standard end to side graft. C) "Patch" graft D) Spatulated end to side graft facing cephalad



Fig 4 Renal angiogram in the dog 1 year after placing a "patch" graft (See arrow) Note the graft's similarity to the normal renal artery—aortic takeoff.

In order to evaluate the effectiveness of an arterial graft not only must anatomic results be known but also their physiologic effectiveness to transmit blood Since it is possible that there may be a significant difference in stricture formation and in blood flow in the grafts described renal angiography (Fig 4) autopsy and blood flow studies were performed Blood flows were studied by exposing the lower abdominal aorta and suturing the grafts in series on the aorta (Fig 3) and recording individual flows with a Shipley rotameter along with simultaneous femoral arterial pressures The results are recorded in Tables 1 and 2 The above studies indicate that all grafts in an acute experiment transmit equal blood flow and that only the end to side technique resulted in lumenal stenosis The patch graft however was the only graft which always remained patent (Table 1) A renal angiogram performed 1 year after placement of a patch graft is represented in Figure 4

Table 1 Results of Angiogram and Autopsy Studies

TYPE OF GRAFT	NUMBER OF GRAFTS PLACED	PERCENTAGE OF PATENCY AFTER 6 MONTHS	AMOUNT OF LUMENAL STENOSIS AFTER 6 MONTHS
End to side	3	66	0 5 mm
Spatulated end to side graft			
a Facing caudad	3	33	None
b Facing cephalad	4	All thrombosed	None
Aortic segment	2	50	None
Patch graft	4	100	None

Table 2 Blood Flow Studies

TYPE OF GRAFT	BLOOD FLOW IN CC/MIN
End to side graft	158
Spatulated end to side graft	
a Facing caudad	[illegible]
b Facing cephalad	[illegible]
Patch graft	[illegible]
Normal renal artery	160

A patch graft is a homograft obtained by excising the renal artery with a 3 to 4 mm margin of aortic wall (Fig 2) It may be preserved by any of the usual methods This graft has several very important features Anatomically there is a natural funneling mechanism present in all arteries leading from the aorta this mechanism is preserved in the patch graft (Fig 2) Because the renal arteries normally curve cephalad as they

branch from the aorta (Fig 2) the "patch" graft may be applied to the aorta *below* the renal artery, eliminating renal ischemia during the aortic graft anastomosis The margin of aortic wall excised when the graft is obtained serves two functions: 1) during the aortic graft anastomosis, the lumen edge is *not handled or traumatized*, and 2) the suture line is 2 to 3 mm removed from the actual graft lumen, making the chances of lumenal stenosis remote and thrombosis due to trauma minimal

METHOD

Technique of Application. The aorta is exposed and mobilized slightly above and below the renal artery About 5 cm of aorta *below* the renal artery is isolated between atraumatic arterial clamps An elipse, 1½ cm to 2 cm long and 5 to 8 mm wide is removed from the aorta Mattress sutures are placed at each end of the "patch" and into the aorta to secure the graft (Fig 1) Care is exercised to place the initial sutures so that the natural funnel of the renal artery (on the graft) faces the aortic blood flow as it does normally After the graft is secured, the anastomosis is completed with the usual everting whip stitch The distal anastomosis is accomplished by standard spatulated end to-end technique

DISCUSSION

Our experimental studies indicate that the "patch" graft fills the requirements of an ideal renal artery graft, that is, ease of application, minimal renal ischemia during its application, adequate blood flow postoperatively, no lumenal stenosis, minimal chance of thrombosis, and anatomic and physiologic identity with the normal renal artery aortic takeoff For unilateral disease we believe the "patch" graft to be the method of choice for renal artery replacement

SUMMARY

1 Experimentally, the "patch" graft is superior to other methods for renal artery replacement

2 The technique for obtaining and applying the graft is described

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SEPARATED KIDNEY FUNCTION TESTS IN HYPERTENSIVE PATIENTS *

EUGENE F POUTASSE ANDREW DONNELLY, AND HARRIET P DUSTAN

Occlusive disease of the renal arteries or their major branches is a significant cause of *hypertension* *Recognition of such lesions* is important since the associated hypertension usually remits following appropriate renal surgery At the present time the best diagnostic technique is renal angiography by the translumbar approach, this in our hands is a safe procedure Because of the potential hazards of angiography other diagnostic methods have been sought In 1957 Connor *et al* [1] *suggested that comparative measurements of* volume and sodium concentration of ureteral urine specimens might aid in finding those kidneys responsible for hypertension in patients with unilateral renal disease In four of their patients whose hypertension had remitted following nephrectomy, preoperative examination had revealed that the affected kidneys put out urine of smaller volume and lower sodium concentration than their mates

We have studied the functions of the individual kidneys of hypertensive patients with essential hypertension pyelonephritis and angiographically proven renal arterial lesions The purpose of this report is to describe the renal functional characteristics found in these patients as regards urine flow, glomerular filtration rate and urine sodium concentration

METHOD

The prerequisite for this test is the quantitative simultaneous collection of urine from each kidney To accomplish this one ureter is occluded by a #10 Garceau catheter at 5 to 8 cm The urine is collected from the other kidney through the water valve of the #24 operating cystoscope The *cystoscope and ureteral catheter are left in place throughout* the test Initially urine flow from the ureteral catheter may be absent but usually peristaltic activity returns in a few moments and with a normal kidney there is a spurt of urine regularly every fifteen to twenty seconds under the conditions of this test To test that the ureter is completely occluded 2 or 3 ml of diluted methylene blue are injected into the ureteral catheter and if none appears in the urine collected from the cystoscope ureteral occlusion is assured If methylene blue appears the ureteral catheter must be adjusted or replaced by one *with an inflatable balloon*

To provide adequate rates of urine flow and to stabilize sodium excretion the tests are performed during osmotic diuresis using mannitol as the loading solute Pitressin infused simultaneously serves as the stimulus for water reabsorption Twenty per cent mannitol is used and to this pitressin is added The *priming infusion* contains a volume of 20% mannitol sufficient to supply an additional 20 mOsm /L to the extracellular fluid—estimated as 20% of the body weight—and 100 milliunits of pitressin This solution is given at the rate of not less than *20 ml /min* and is followed immediately by the *sustaining infusion* which delivered at a rate of 3 ml /min ,

* From the Department of Urology and Research Division of the Cleveland Clinic Foundation and the Frank F Bunts Educational Institute Cleveland

is allowed to run throughout the test. This solution provides approximately 3 mOsm of mannitol and 1.5 milliunits of pitressin/minute. The priming infusion is begun at least 30 minutes prior to the cystoscopy. To allow for equilibration of the mannitol in the body fluids, the sustaining solution must start at least 20 minutes prior to urine collection.

Ureteral specimens are collected simultaneously from each kidney during two 15 minute periods; a blood specimen is drawn at the midpoint of each period. The following renal functions are measured: (1) urine flow (V) expressed as ml./min./1.73 M^2; (2) glomerular filtration rate (GFR) calculated from the clearance of mannitol; and (3) urine sodium concentration (U_{Na}). The analytical procedures used were those previously described.[7]

This test was performed on 33 hypertensive patients, all of whom had had renal angiography. Eleven were considered to have essential hypertension; 6, pyelonephritis—predominately unilateral; 6, unilateral renal arterial disease; 6, bilateral renal arterial disease; and 4, disease of a segmental branch of one renal artery.

RESULTS

Urine Flow and Glomerular Filtration Rate (Table 1). In each of the 11 patients with essential hypertension the urine flow (V) was practically identical on the two sides. Minor differences were found; the greatest difference was 0.4 ml./min., but in the remaining 10 patients V on one side was not more than 0.25 ml. different from that of its mate. Glomerular filtration rates (GFR) of the two kidneys were likewise similar in all these patients. The differences observed between the two sides were minimal and were usually less than 10%.

The patients with pyelonephritis and renal arterial lesions were found to have inequalities of urine flow which reflected similar differences in GFR. Regardless of the nature of the lesion which had led to a depression of GFR, this depression was consistently expressed by a lower rate of V. As a general rule the difference in V was at least 2 ml./min. and often was considerably greater; however, in 3 patients with bilateral renal arterial lesions differences of 0.5 to 1.0 ml./min. reflected significant differences in GFR.

Urine Sodium Concentration. The urine sodium concentration (U_{Na}) on the two sides was practically identical in the patients with essential hypertension, pyelonephritis, and occlusive disease of a segmental branch of one renal artery. In these patients U_{Na} ranged from 23 to 101 mEq./L.; minor differences in concentration between the two sides were observed but these were usually less than 5% and did not exceed 11%.

Consistent depressions of U_{Na} characterized the urine from the affected side in the 6 patients with unilateral renal arterial lesions. The U_{Na} on the unaffected side ranged from 55 to 91 mEq./L. and on the affected side 12 to 57 mEq./L. Depressions of more than 20% were found in each patient. Thus, in these patients, changes in U_{Na} paralleled changes in V and GFR.

In 3 of the 6 patients with bilateral renal arterial lesions depressions of U_{Na} were found on the sides which also had lower rates of V and GFR; in one patient U_{Na} on the two sides were identical, while in the remaining two U_{Na} was higher on the side of the lower V and GFR.

Table 1 Relationship of Urine Flow (V) to Glomerular Filtration Rate (GFR) in Patients with Essential Hypertension, Pyelonephritis and Renal Arterial Lesions

	KIDNEY A		KIDNEY B	
	V	GFR	V	GFR
	ESSENTIAL HYPERTENSION			
Means for 11 Patients	3.83	42	3.80	44
PATIENT NO	PYELONEPHRITIS			
1	5.2	18	1.9	18
2	4.5	41	2.7	24
3	12.0	86	5.5	24
4	12.4	59	1.8	8
5	8.8	73	3.0	24
6	6.0	51	7	6
	ARTERIAL BRANCH LESION			
7	9.3	76	4.2	32
8	9.8	83	7.4	67
9	1.9	52	3.6	34
10	7.4	89	2.4	31

PATIENT NO	KIDNEY A V	KIDNEY A GFR	KIDNEY B V	KIDNEY B GFR
	BILATERAL ARTERIAL LESIONS			
11	7.5	87	1.3	31
12	2.1	19	2	1.3
13	3.2	56	2.8	46
14	3.6	61	2.6	49
15	3.2	42	2.3	31
16	13.7	70	2.9	32
	UNILATERAL ARTERIAL LESION			
17	3.3	59	2.2	[illegible]
18	4.9	69	2.0	42
19	7.6	71	1.0	12
20	5.0	71	2.1	41
21	7.9	89	2.7	48
22	10.6	75	1.9	33

DISCUSSION

Our results show that separated renal function tests are of value in the investigation of patients with hypertension since they can show disparity in renal function between the two sides. The conditions of the test (osmotic diuresis and pitressin infusion) are such that V must be dependent upon GFR and in situations where the measurement of GFR is not possible determination of V alone can therefore give valuable information. The reliability of the association between these two functions is emphasized by our results in the patients with essential hypertension. In these V and GFR were similar on the two sides; such findings are in accord with the earlier observations of Chasis and Redisch[3] that nephrosclerosis does not lead to disparities in renal function. Similarities in V and GFR establish that the use of an occlusion catheter does not alter renal function on that side. Thus when simultaneous ureteral urine collections are accurately performed in hypertensive patients, differences in V between the two sides indicate disparities in renal function that may suggest a causal factor in the patient's hypertension.

Although these tests were performed during mannitol diuresis with pitressin antidiuresis, similar dependence of V upon GFR would be expected also during water diuresis.[4]

Differences in U_{Na} between the two sides can be produced by main renal artery lesions if these are *located proximal to the point of segmental branching*. In our patients with unilateral lesions U_{Na} was depressed on the side of the lesion and accordingly paralleled the observed decreases in V and GFR. With only these data available one would conclude that depressions of V and U_{Na} signify a unilateral arterial lesion. If such changes were caused exclusively by unilateral lesions, comparative measurements of V and U_{Na} in ureteral urine specimens would be expected to indicate which of two kidneys is responsible for hypertension. Unfortunately these parallel depressions of V and U_{Na} were also found in 3 of 6 patients with bilateral arterial lesions—a clinical situtation in which uninephrectomy would be foolhardy. Another point concerning the diagnostic importance of U_{Na} changes bears emphasis: a lesion of segmental branch of one renal artery—with or without partial renal infarction—does not change renal sodium handling and there is no disparity of U_{Na} in the ureteral urine specimens. This type of renal vascular lesion can be associated with severe hypertension that remits following nephrectomy but the results of the function tests cannot differentiate between this lesion and pyelonephritis. Thus without renal angiography, undue emphasis on parallel depressions of V and U_{Na} can, on the one hand, lead to an erroneous diagnosis of a unilateral arterial lesion and on the other fail to indicate a segmental branch lesion. In short, our experience with separated renal function tests in patients with renal vascular lesions has but strengthened our earlier opinion[5] that renal angiography is at the present time the best way to detect occlusive disease of the renal arteries or their major branches. Whereas renal function tests cannot substitute for angiography they can indicate those patients in whom angiography should be performed.

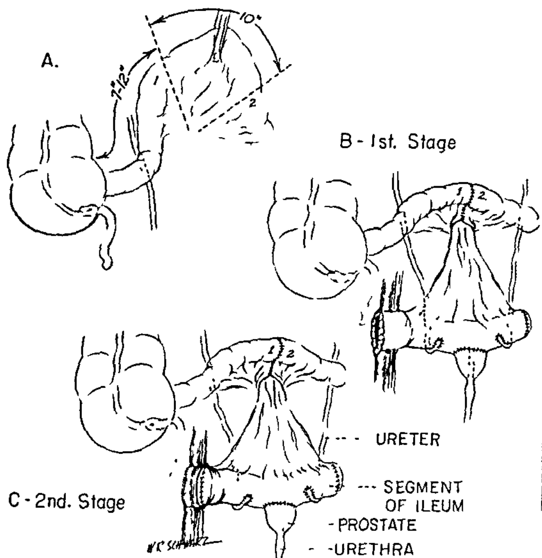


Fig 1 Diagrammatic outline of the two stage procedure A Segment of ileum to be isolated B ureteral and urethral anastomosis C cutaneous opening of ileum closed extraperitoneally

occurred The fatalities were due to acute pylonephritis following repeated dilations of a stenosing external urethrotomy, overdose of sodium pentothal, trauma, and intraperitoneal perforation of a prostatic abscess respectively All 5 surviving animals received the specially devised urteral anastomosis Following closure of the cutaneous openings of the segments continent voiding without residual began immediately Cystometrograms performed on 3 of the animals revealed bladder capacities of about 120 cc with forceful kickoff at volumes of from 100 to 120 cc Cystograms revealed evidence of reflux in 2 animals on one side only Chemistries remained normal A striking fact was that after 3 to 6 weeks very little mucus was produced, this prompting closure of the cutaneous openings of the loops After closure mucus formation was still not a problem The animals were sacrificed at intervals of from 3 to 7 months and practically no mucus was found No gross pathological changes were noted

Histological studies, though as yet incomplete, revealed a consistent change

in the mucosa of the substitute bladders consisting of a pseudomembranous type of necrosis with numerical diminution in glandular structures The basement membranes remained intact, viable and free of inflammatory changes

Two Stage Procedure in Man. On the basis of our experimental work we felt justified in performing the two stage operation upon a 38 year old white male, who for 10 years had progressive leukoplakia of the urinary bladder with scattered islands of cystitis glandularis and a bladder capacity of from 30 to 10 cc. The patient is 9 months postoperative, mucus is no problem, and there is continent voiding without residual. Excretory urograms are completely normal and the urine is sterile Cystograms, however, reveal some reflux up the right side Cystometrogram reveals forceful kickoff at 175 cc.

SUMMARY AND CONCLUSION

The two stage procedure appears to be the operation of choice because of the mucus factor. Optimum time to close the cutaneous opening is when the mucus secretion has diminished, chemistries have stabilized, and dilation of the upper urinary tract has subsided. The specially devised ureteral anastomosis seems superior in that a blunt end to side junction avoids stricture formation while its long attachment to the segment affords a somewhat sphincteric action The peculiar pseudomembranous necrosis of the mucosa is clinically beneficial in that there is corresponding decrease in the mucous secretion Realizing that these studies are far from complete, 1 survivor of the two stage procedure and 4 newly prepared animals are being studied for alterations occurring over a long period of time

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SUBSTITUTE URINARY BLADDER CONSTRUCTED FROM AN ISOLATED SEGMENT OF SMALL BOWEL *

S R WEINBERG, K WATERHOUSE, F SALERNO, AND F C HAMM

Lack of a satisfactory bladder substitute has been the principal deterrent to cystectomy for malignancy of the bladder This study is concerned with the use of an isolated small bowel segment so constructed as to form not merely a conduit for urine but a reservoir After cystectomy, it is attached to the urethra so that voiding is through natural channels

The initial experiment that utilized an isolated ileac segment as a bladder

* From the State University of New York Downstate Medical Center Department of Surgery Division of Urology Brooklyn Supported by U S Public Health Grant #H2908 (C1) and American Cancer Society Grant #T 96

dates back to Tizzoni and Foggi in 1888,[1] but in more recent times it has been revived by Couvelaire of Paris.[2] Cibert and Couvelaire[3] have reported 42 cases, Gertz and Franksson[4] of Stockholm have reported 19 cases while Pyrah and Raper[5] of Leeds have presented 3. It must be noted that the operative mortality in these series was high—e.g. 7 deaths in 21 cases done for infiltrating carcinoma (Cibert) 5 deaths in 16 cases (Giertz).

METHOD

Initially our efforts to form a bladder from ileum were in animals. The surgery as done in the dog consisted of isolating a segment of small bowel 10 cm. long and 18 cm. from the ileocaecal valve and then restoring the continuity of the intestine. The proximal part of the isolated segment was then incised on its antemesenteric border and sutured to itself in such a manner as to form a pouch (Fig. 1, 2, 3, 4). The lower end of ileac segment was left intact for a distance of 4 cm. After the intestinal pouch had been formed, the bladder was removed and the ureters were anastomosed to the straight distal part of the new bladder just below the pouch portion. A catheter was then passed into the urethra and with this as a guide the distal end of the ileac segment was anastomosed to the urethra.

The procedure was carried out in 13 female adult dogs. In 8 of the animals the procedure was done in two stages. In the first stage the new viscus was constructed and allowed to drain to the skin. In the second stage the cystectomy and ureteroileourethroplasty was completed. In the remaining 5 the procedure was done in one stage. Results of the procedure fall into three groups. Six animals died in the immediate postoperative period of peritonitis due to extravasated urine, 3 died 2 to 3 weeks later of intestinal obstruction.

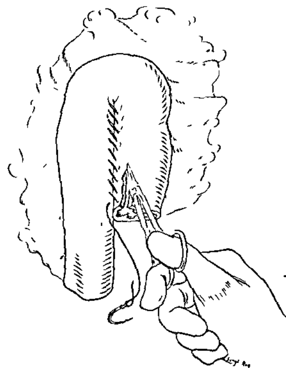


Fig 1 Loop of ileum folded and sutured to itself except for distal 4 cm

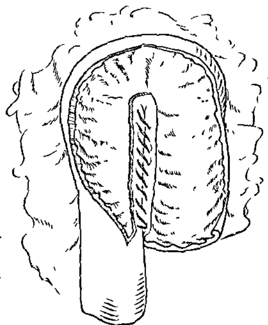


Fig 2 Double barreled loop opened along antemesenteric border.

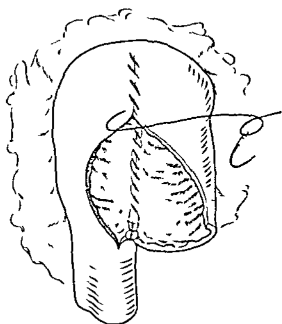


Fig 3 Beginning of anterior closure

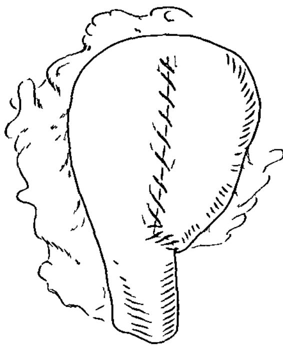


Fig 4 New Bladder in its final form Note unaltered distal 4 cm of ileac loop which is attached to urethra and into which the ureters are implanted

1 animal died 5 months after the operation because of bilateral hydronephrosis At the time of writing the remaining 3 animals are continent and appear to be in good health 8 to 10 months after the operation Excretory urograms show normal renal function and morphology in 1 kidney of each dog but some degree of ureteral obstruction in the other The blood chemistries of 3 survivors on 8–27–58 are shown in Table 1

It is apparent from these values that all the animals have some degree of nitrogen retention and acidosis

Table 1

DOG	BUN MG%	CO^2 MEQ /L	SODIUM MEQ /L	POTASSIUM MEQ /L	CHLORIDES MEQ /L
Normal values	10 20	18 7 22 0	135 160	3 7 5 8	99 110
1167	40	11 6	—	—	111
838	38	17	140	5 1	115
543	34	15.2	144	4 6	111

Despite the poor experimental results the operation has been done for 3 patients because the two principal causes of failure in the dog would not apply to the human subject Anastomosis of the ureter to small bowel in the dog is difficult because of the small size of the dog ureter and the relative thickness of the dog ileum More important the peritoneum in the dog is so thin that the posterior layer cannot be sutured to the anterior layer leaving the uretero ileac anastomoses in the peritoneal cavity In the human on the other hand

the anastomoses can be placed extraperitoneal so that any extravasated urine can be drained Furthermore the vascular pedicle of the isolated bowel segment in the dog is relatively longer and cordlike and so more prone to obstruct an intestinal loop In man the pedicle is fan shaped and can be placed largely extraperitoneal

One can rightly question whether deleterious effects will follow placing the total urinary output into a pouch lined by intestinal mucous membrane Our previous experience with ileocystoplasty [6] however led us to believe that a urine reservoir composed solely of ileum would be able to store and discharge urine satisfactorily and cause neither metabolic acidosis nor persistent urinary tract infection In the animals studied 80% of the bladder had been replaced by ileum and long follow up studies showed no alteration of blood chemistry 10 months after the operation Likewise our experience with 6 patients who had undergone ileocystoplasty for contracted bladders and had been left essentially with only the trigone of the bladder remaining was also satisfactory

In the human subject the operation has been done through a transverse suprapubic incision The recti muscles were cut from the symphysis pubis (Czerny) The peritoneum was incised transversely just above its attachment to the bladder facilitating subsequent closure of the peritoneal cavity at the end of the operation and allowing the anastomotic sites to be placed extraperitoneally Prior to the cystectomy and formation of the ileac bladder the internal iliac arteries were ligated The isolated intestinal segment was taken 18 cm from the ileocecal valve and was 36 cm in length the proximal 24 cm was folded on itself to form the pouch leaving a distal 2 cm to be anastomosed to the urethra Before closing the peritoneal cavity the entire operative site was irrigated with a solution of Chlorpactin XCB [7]

The first patient (MG KCH #7724) a female age 53 had a large sessile tumor on the right lateral wall and 2 smaller similar tumors on the left side of the bladder Biopsy showed medium grade of malignancy Cystectomy and ureteroileourethroplasty was done March 13 1958 The ureters were anastomosed to the isolated ileac segment by the Nesbit technique The postoperative course was uneventful except for phlebitis The prevesical space was drained by a rubber tissue drain for 12 days Urinary drainage persisted for 5 weeks but an open sinus prevented pooling of urine and the urethral catheter was kept indwelling until there was healing The patient was discharged May 12 1958 At that time she was able to void with some abdominal straining There is stress incontinence The postoperative excretory urogram taken 1 month after operation was satisfactory as well as the postoperative blood studies BUN 20 and creatine 1 3 mg % CO_2 22 2 chlorides 105 K 314 mEq /L Examination of the removed bladder showed that the tumor had invaded half way through the muscle layer of the bladder

The second patient (D G LICH #102350) a 44 year old female had a 5 year history of recurrent bladder papillomata For the first 3 years recurrences were benign but the later growths were malignant The patient tolerated the operation (7 21 58) without incident She was ambulatory and discharged on 8 5-58 The patient had no disturbance of renal function postoperatively Blood chemistry 4 days after operation was normal BUN 20 mg % chlorides 90 mEq /L and sodium 136 mEq/L When the catheter was removed 3 weeks postoperative urinary drainage reappeared so that the catheter was reinserted but could be removed after 5 weeks The patient is now voiding bladder

capacity is 175 cc residual urine 30 cc and the urine is clear except for mucous The patient is at home ambulatory and without major complaints

The surgery done for our third patient a male age 58 (R M KCH #31653) is still too recent for complete analysis This patient was operated on for multiple extensive areas of transitional cell carcinoma of medium grade The operation was not essentially different from those done in females except that this was a secondary procedure done after the patient had had a cystotomy following rupture of the bladder during transurethral resection of the bladder tumors At the time of writing the patient still has an indwelling urethral catheter

It must be noted that lavage of the new bladder is important early in the postoperative period as blood and mucous can close the eyes of the urethral catheter and create a suprapubic urinary sinus that is difficult to close None of these patients had elevation of temperature or any other serious signs of urinary tract infection The urine contained mucous but not an amount sufficient to cause obstruction or discomfort

SUMMARY

A pouch can be constructed from an isolated ileac segment to simulate the urinary bladder Because the procedure is lengthy and of considerable risk its use has been restricted to patients with multiple and extensive areas of malignancy where cystectomy may be curative rather than palliative As the work is continuing no final evaluation can be made At this time the procedure must be considered of an experimental nature and we are apprehensive that failure of proper emptying of the reconstructed bladder may cause damage to the upper urinary tract

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EVALUATION OF A SUBSTITUTE DETRUSOR *

JOHN J MURPHY WILLIAM H RATTNER AND WILLIAM S BLAKEMORE

In certain clinical conditions encountered in urologic practice the detrusor muscle of the urinary bladder is found to be useless while the trigone bladder neck and urethra remain relatively normal Chronic overdistention of the bladder with superimposed infection is perhaps the most common cause of these findings while prolonged cystostomy tube or catheter drainage with secondary infection congenital anomalies and certain neurologic syndromes are also known to produce them Since the damage appears to be limited to the detrusor muscle itself replacement of this damaged tissue by normal muscle provides an appealing approach to the treatment of this problem Recent interest in various types of enterocystoplasty suggests that segments of bowel attached to damaged bladders may improve voiding function The purpose of this investigation was to measure the pressures which can be developed in an isolated segment of bowel and to determine clinically the feasibility and efficiency of such a segment when used as a substitute detrusor

METHOD

Three patients were selected whose detrusor muscle appeared to have been rendered functionless or grossly inefficient by disease The remainder of the bladder including the trigone outlet and urethra were within normal limits Studies included intravenous urography urethrocystoscopy cystometry cystogram in some cases cinefluorography and measurement of pressures in the intact bowel and isolated segment by means of a catheter and strain gauge manometer

Operative Technique The bladder was exposed transperitoneally The peritoneum was removed from the superior and posterior surfaces and the bladder completely freed up to the trigone and bladder neck A segment of sigmoid colon was selected which could be brought easily to the trigonal area and this segment was isolated with mesentery intact The length of the segment varied between 7 and 9 inches Continuity of the colon was restored by an end to end anastomosis The proximal end of the isolated segment was inverted and closed with two layers of catgut and one of silk The bladder was then opened and the detrusor muscle resected leaving only the bladder neck and trigone intact The distal end of the isolated segment was sutured to the trigone and bladder outlet This required an incision along the anterior edge of the isolated segment in order to permit anastomosis to the outlet (Fig 1) Postoperatively the new bladder was drained by means of a suprapubic cystostomy tube as well as an indwelling urethral catheter The urethral catheter was removed as soon as the urine was clear and the cystostomy tube was removed between the 10th and 14th days

Three patients have been operated upon by this technique Postoperative studies included intravenous urography urethrocystoscopy cystogram cinefluorography cystometry and cystometry by means of the strain gauge manometers Except for minor complications the clinical course of each of the patients was satisfactory

* From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia

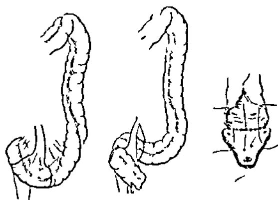


Fig 1 Illustrates the construction of the sigmoid bladder and its anastomosis to the trigone and vesical neck

The studies of a representative patient are demonstrated in the following figures preoperative and postoperative intravenous urogram (Fig. 2 A & B), preoperative cystogram and postoperative cystogram (Fig. 3 A & B), preoperative cystometry and postoperative cystometry (Fig. 4 A & B) including residual urines Measurements of the pressure of the intact bowel with strain gauge manometers and measurement by similar technique of the pressures developed within the substitute detrusor after anastomosis of the isolated segment of the trigone are shown in Figure 5 A & B.

This patient voids every 2 to 4 hours with a slow but wide stream, and apparently empties well The urine contains a moderate amount of mucus and numerous white blood cells, but there are no clinical signs or symptoms.

CONCLUSIONS

It appears that pressures in the intact colon may be increased when one end is closed and the isolated segment is used to substitute for the bladder detrusor muscle. Maximum pressure developed by such a segment without straining

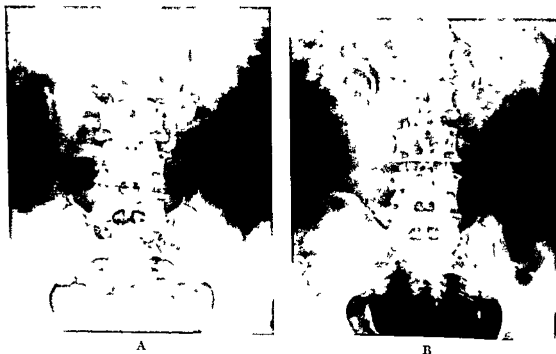


Fig 2 (A) Preoperative and (B) Postoperative urograms on H J, demonstrating a normal upper urinary tract



Fig 3 (A) Preoperative and (B) postoperative cystograms on H J The preoperative cystogram demonstrates a large redundant bladder The postoperative cystogram shows the sigmoid bladder filled

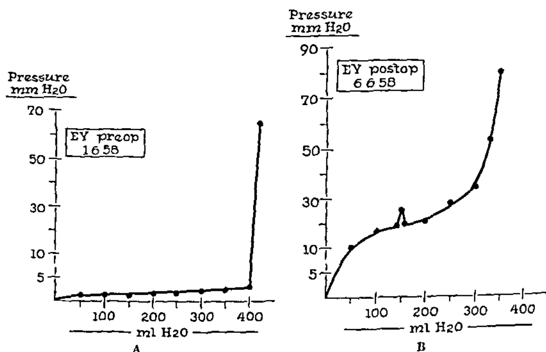


Fig 4 (A) Preoperative and (B) postoperative cystometrograms (E V) The residual urine prior to surgery was 350 cc Following surgery the residual urine was 100 cc The maximum pressures were recorded while the patient was straining to increase the pressure with her abdominal muscles

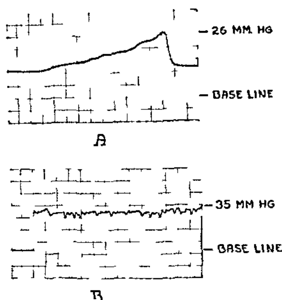


Fig 5 (A) Maximum pressure recorded by a strain gauge manometer of the intact sigmoid colon (J L) (B) Maximum pressure recorded by the strain gauge manometer of the sigmoid bladder without straining

did not exceed 50 cm of water but could be augmented with straining to more than 90 cm of water Except in the case of female patients who have undergone resection of the bladder neck and in males who have had prostatic resection this pressure may be inadequate to permit emptying

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ASSESSMENT OF THE ROLE OF THE KIDNEY, LIVER AND COLONIC MUCOSA IN HYPERCHLOREMIC ACIDOSIS *

An Experimental Study

Ronald H Hayward Khalil G Wakim, William H ReMine
John H Grindlay and Jesse L Bollman

It is known that a state of hyperchloremic acidosis may develop following diversion of urine into the colon This is a preliminary report of an investigation of this problem in dogs The objectives of the study were (1) to test the relative importance of renal pathology and reabsorption of electrolytes in the production of hyperchloremic acidosis after ureterocolic anastomosis (2) to appraise the importance of and the factors contributing to depletion of potassium, (3) to determine whether functional or histologic renal damage occurs following prolonged urinary diversion in the absence of gross kidney damage such as might result from ureteral obstruction and (4) to learn the effect of preventing the reabsorbed urinary products from reaching the liver by diverting the portal blood from the colon receptacle into the systemic venous circulation

* From the Mayo Foundation and the Mayo Clinic Rochester Minnesota



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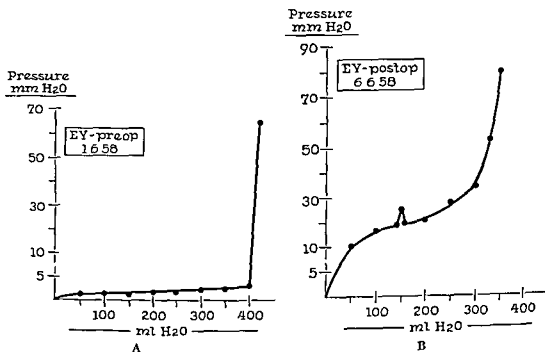


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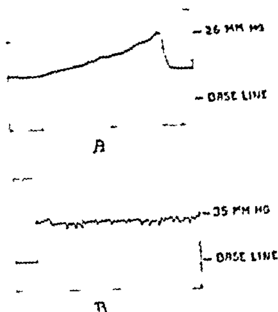


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* From the M... F...

THE ROLE OF THE COLONIC MUCOSA

In obtaining colonic drainage of urine, because ureterosigmoidostomy involves a hazard of ureteral obstruction with subsequent hydronephrosis, we chose to anastomose to the colon either the whole bladder or the bladder trigone with a surrounding cuff of bladder wall In one group of dogs the anastomosis was made to the rectum (low) and in another group to the region of the cecum (high), and in a number of animals an intermediate site was used The results were followed carefully, in some instances for 12 months by repeated recording of body weight and analysis of blood to determine the presence and rate of development of imbalance of electrolytes or acid base Blood alkali reserve, pH, and concentrations of urea, chloride, and potassium were determined frequently, and at certain intervals determinations were made also of the concentrations of sodium, creatinine, phosphate, magnesium, ammonia, and erythrocytes At necropsy, sections of the colon were taken for histologic study and the level of the site of anastomosis was measured for calculation of the percentage area of the colonic mucosa which had been exposed to urine

Those dogs with diversion of urine into the rectum which exposed less than 40% of the colonic mucosa maintained a normal acid base balance throughout the experiment and gave evidence of only moderate hyperchloremia (Table 1) These animals maintained or gained weight Their serum potassium levels were not low, and at the end of the experiment their muscle potassium levels were normal

Table 1 Representative Concentrations of Blood Factors in Dogs With Diversion of Urine Into Rectum

Sodium	147 mEq /L
Potassium	5 mEq /L
Chloride	111 mEq /L
Urea	80 mg /100 ml
Phosphate	4 mg /100 ml
Creatinine	1 mg /100 ml
Alkali reserve	25 mM /L
pH	7 30

Where the anastomosis was made to expose 60 to 70% of the colonic mucosa to urine a state of moderate acidosis was usually found

Where up to 100% of the colonic mucosa was exposed hyperchloremia and severe acidosis usually developed within 10 to 14 days (Table 2) Comparison with the findings from the first group shows a definite elevation of chloride and a definite reduction in pH and alkali reserve These dogs with an anastomosis to the proximal colon did poorly, as a rule, and most of them lived only 6 to 8 weeks The cause of death was uncertain but it did not appear to be due to excessive acidosis hyperchloremia or potassium deficiency although the serum potassium levels fell progressively with longer survival *Electrocardiograms* did not support a diagnosis of potassium depletion, and at death muscle potassium determinations were within the normal range except in one instance Two dogs did show definite improvement after administration of

Table 2 Representative Concentrations of Blood Factors in Dogs With Diversion of Urine Into Proximal Colon

Sodium	147 mEq /L
Potassium	4.8 mEq /L
Chloride	118 mEq /L
Urea	80 mg /100 ml
Phosphate	4 mg /100 ml
Creatinine	1 mg /100 ml
Alkali reserve	15 mM /L
pH	7 15

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THE ROLE OF THE KIDNEY

Excretory urograms were performed at intervals to determine the presence of ureterectasis and pyelocaliectasis Signs of obstruction were absent or minimal in the majority of cases, and there was no obvious difference between the individuals showing acidosis and those with a normal acid base balance In 2 dogs with anastomosis of the bladder to the rectum, gross dilatation of the ureters and renal pelves developed because of a technical error, but did not lead to excessive hyperchloremia or acidosis In several dogs with anastomosis to the proximal colon, excretory urograms were made prior to operation and again 10 to 14 days later, after acidosis had developed, but there was no concomitant change in the roentgenographic picture to explain the change in acid base balance

Several dogs with each type of bladder anastomosis were subjected to unilateral nephrectomy and partial destruction of the remaining kidney The latter procedure was accomplished by removing plugs of renal tissue with a punch and filling the defects with gelfoam After the resultant infarction and scarring of a portion of the kidney, the remainder underwent compensatory hypertrophy and became the only functioning renal tissue The hematologic findings in these dogs did not vary significantly from those in others with a similar type of urinary diversion

At the termination of the experiment, in order to assess the functional status of each dog s kidneys, bilateral (or unilateral if there was only one kidney) nephrostomy was performed with aseptic precautions Polyvinyl tubing was inserted up the ureter in a retrograde fashion and exteriorized through the kidney substance and abdominal wall The ureter was ligated and divided below the flanged end of the tube, and avulsion of the latter was prevented by fixation to the renal capsule and parietal peritoneum with polyvinyl (ivalon) sponge

At the time of this procedure samples of urine were removed directly from the renal pelves for bacterial culture Growths of Escherichia coli, Aerobacter aerogenes or Streptococcus faecalis were found in nearly all cases It is apparent, therefore, that bacterial contamination of the urine almost always follows anastomosis of the bladder to the colon

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THE ROLE OF THE COLONIC MUCOSA

In obtaining colonic drainage of urine, because ureterosigmoidostomy involves a hazard of ureteral obstruction with subsequent hydronephrosis we chose to anastomose to the colon either the whole bladder or the bladder trigone with a surrounding cuff of bladder wall In one group of dogs the anastomosis was made to the rectum (low), and in another group to the region of the cecum (high) and in a number of animals an intermediate site was used The results were followed carefully, in some instances for 12 months, by repeated recording of body weight and analysis of blood to determine the presence and rate of development of imbalance of electrolytes or acid base Blood alkali reserve, pH, and concentrations of urea, chloride, and potassium were determined frequently, and at certain intervals determinations were made also of the concentrations of sodium, creatinine, phosphate, magnesium, ammonia, and erythrocytes At necropsy, sections of the colon were taken for histologic study and the level of the site of anastomosis was measured for calculation of the percentage area of the colonic mucosa which had been exposed to urine

Those dogs with diversion of urine into the rectum, which exposed less than 40% of the colonic mucosa, maintained a normal acid base balance throughout the experiment and gave evidence of only moderate hyperchloremia (Table 1) These animals maintained or gained weight Their serum potassium levels were not low, and at the end of the experiment their muscle potassium levels were normal

Table 1 Representative Concentrations of Blood Factors in Dogs With Diversion of Urine Into Rectum

Sodium	147 mEq /L
Potassium	5 mEq /L
Chloride	111 mEq /L
Urea	80 mg /100 ml
Phosphate	4 mg /100 ml
Creatinine	1 mg /100 ml
Alkali reserve	25 mM /L
pH	7 30

Where the anastomosis was made to expose 60 to 70% of the colonic mucosa to urine a state of moderate acidosis was usually found

Where up to 100% of the colonic mucosa was exposed, hyperchloremia and severe acidosis usually developed within 10 to 14 days (Table 2) Comparison with the findings from the first group shows a definite elevation of chloride and a definite reduction in pH and alkali reserve These dogs with an anastomosis to the proximal colon did poorly, as a rule, and most of them lived only 6 to 8 weeks The cause of death was uncertain, but it did not appear to be due to excessive acidosis, hyperchloremia, or potassium deficiency, although the serum potassium levels fell progressively with longer survival Electrocardiograms did not support a diagnosis of potassium depletion, and at death muscle potassium determinations were within the normal range except in one instance Two dogs did show definite improvement after administration of

Table 2 Representative Concentrations of Blood Factors in Dogs With Diversion of Urine Into Proximal Colon

Sodium	147 mEq /L
Potassium	4 8 mEq /L
Chloride	118 mEq /L
Urea	80 mg /100 ml
Phosphate	4 mg /100 ml
Creatinine	1 mg /100 ml
Alkali reserve	15 mM /L
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Several dogs with each type of bladder anastomosis were subjected to unilateral nephrectomy and partial destruction of the remaining kidney The latter procedure was accomplished by removing plugs of renal tissue with a punch and filling the defects with gelfoam After the resultant infarction and scarring of a portion of the kidney, the remainder underwent compensatory hypertrophy and became the only functioning renal tissue The hematologic findings in these dogs did not vary significantly from those in others with a similar type of urinary diversion

At the termination of the experiment, in order to assess the functional status of each dog s kidneys, bilateral (or unilateral if there was only one kidney) nephrostomy was performed with aseptic precautions Polyvinyl tubing was inserted up the ureter in a retrograde fashion and exteriorized through the kidney substance and abdominal wall The ureter was ligated and divided below the flanged end of the tube, and avulsion of the latter was prevented by fixation to the renal capsule and parietal peritoneum with polyvinyl (ivalon) sponge

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Table 2 Representative Concentrations of Blood Factors in Dogs With Diversion of Urine Into Proximal Colon

Sodium	147 mEq/L
Potassium	4.8 mEq/L
Chloride	118 mEq/L
Urea	80 mg/100 ml
Phosphate	4 mg/100 ml
Creatinine	1 mg/100 ml
Alkali reserve	15 mM/L
pH	7 15

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Tests of kidney function were performed using urine collected via the nephrostomy tubes From the first postoperative specimen it was possible to determine the specific gravity of the urine and the ability of the kidney to produce ammonia and urine of high acidity On the day of the operation the clearance of endogenous creatinine was measured and from this were derived the quantities of chloride and potassium that were filtered reabsorbed by the tubules and excreted in the urine Several days after nephrostomy the latter tests were repeated and followed by a test of urine concentration to determine the maximal specific gravity and urine to plasma ratio of urea that the kidney or kidneys were capable of producing Also a sample of urine was taken for routine urinalysis and was checked particularly for casts and albumin

The tests of renal function performed in dogs from all groups showed no significant change in the ability of the kidney or kidneys to (1) produce acid urine (2) form ammonia (3) remove accumulated urea (4) return the blood chloride to normal (5) retain potassium (6) excrete creatinine (as measured by clearance of endogenous creatinine) and (7) produce urine of high specific gravity and a satisfactory urine to plasma ratio of urea Urinalysis did not reveal albuminuria greater than grade 1 or grade 2 which is normal for the dog and the presence of casts was rare

At necropsy the kidneys looked grossly normal in most instances In some the ureters and renal pelves were moderately dilated but rarely showed signs of inflammation The hypertrophy found in the majority of cases was greater if a large area of colon had been exposed to urine Those dogs subjected to removal of 60 to 70% of renal tissue showed considerable hypertrophy of the remainder which in some cases weighed as much as both kidneys in corresponding dogs of this series Histologic sections were prepared from the kidneys and stained with hematoxylin and eosin Mallory Heidenhain and Sudan IV stains No significant microscopic changes were detected and moderate pyelonephritis was only an occasional finding

THE ROLE OF THE LIVER

In a number of dogs we studied the effect of diverting the colonic venous blood with its reabsorbed urinary products away from the liver To determine the venous drainage of the gastrointestinal tract the portal system was dissected out in several dogs and in 2 of the animals was injected with a mixture of colored gelatin and barium With this method the radicles of the portal vein could be identified by inspection and roentgenography

It was found possible to divert the venous blood in varying degrees by different operative procedures A number of these were performed before the diversion of urine the rest after

In some dogs the portal vein and inferior vena cava were divided and the cut ends transposed These animals did poorly became very toxic and showed raised blood levels of ammonia Although they did not show acidosis the reduced survival time makes it difficult to draw satisfactory conclusions

In others the common mesenteric vein was divided the hepatic end ligated and the caudal end anastomosed end to side to the inferior vena cava Thus all the blood from the gastrointestinal tract beyond the proximal part of the jejunum was diverted These animals also died rapidly unless smallness of the anastomosis limited the diversion

In a third group the anastomosis was similar except that the shunt was made

caudad to the junction of the colonic mesenteric veins with the common mesenteric vein. Thus the colonic venous blood was not diverted from the liver and no alteration of the state of hyperchloremic acidosis was detected.

In 2 dogs in acidosis the principal veins draining the colon were ligated near their junction with the common mesenteric vein, but this procedure did not alter the preexisting hyperchloremia or acidosis and the health of the animals did not deteriorate.

COMMENT

The findings presented in this paper suggest that acidosis may develop rapidly following diversion of urine into the colon, simply from reabsorption of urinary constituents. Renal insufficiency did not play a significant part in the development of hyperchloremic acidosis, for renal function was found to be adequate by all the tests applied, even when 60% of renal tissue had been destroyed. Presumably, hydronephrosis resulting from ureteral obstruction must be severe before the functioning renal tubular mass is reduced beyond a critical point to an inadequate amount. Similarly, it may be assumed that prolonged reabsorption and excretion of urinary products do not lead to deterioration of the kidney.

Loss of potassium may occur in dogs with proximal urinary diversion and probably is due to a combination of two important factors: acidosis and loss of secretions with mucus from the colon. This thought is suggested by the observation that dogs with rectal anastomosis did not become potassium-deficient, nor did a series of animals with acidosis induced by the administration of ammonium chloride. Potassium loss from renal damage was ruled out by demonstrating that the kidneys were able to retain potassium effectively at the end of the experiment.

The results obtained from the portal shunting procedures suggest that the liver itself does not modify the development of hyperchloremic acidosis. It does, however, play an important role in detoxifying reabsorbed urinary products, since diversion of these products to the systemic circulation by portacaval anastomosis is definitely detrimental to the dog. Because of this finding we intend to perform an additional experiment in which the inferior vena cava is ligated so that there will be little if any systemic shunting of portal blood.

CONCLUSIONS

From this investigation in dogs the following tentative conclusions can be made.

1. The onset and severity of acidosis following diversion of urine in the dog is proportional to the area of the colonic mucosa exposed to urine.
2. Acidosis may occur in the absence of renal insufficiency.
3. Prolonged reabsorption of urinary products with or without acidosis does not produce significant kidney damage.
4. Depletion of potassium results mainly from acidosis and the loss of secretions with mucus per rectum.
5. Diversion of portal venous blood with the reabsorbed urinary products into the systemic circulation has a detrimental effect on the animal.

Tests of kidney function were performed using urine collected via the nephrostomy tubes From the first postoperative specimen it was possible to determine the specific gravity of the urine and the ability of the kidney to produce ammonia and urine of high acidity On the day of the operation the clearance of endogenous creatinine was measured and from this were derived the quantities of chloride and potassium that were filtered reabsorbed by the tubules and excreted in the urine Several days after nephrostomy the latter tests were repeated and followed by a test of urine concentration to determine the maximal specific gravity and urine to plasma ratio of urea that the kidney or kidneys were capable of producing Also a sample of urine was taken for routine urinalysis and was checked particularly for casts and albumin

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The findings presented in this paper suggest that acidosis may develop rapidly following diversion of urine into the colon simply from reabsorption of urinary constituents Renal insufficiency did not play a significant part in the development of hyperchloremic acidosis for renal function was found to be adequate by all the tests applied even when 60% of renal tissue had been destroyed Presumably hydronephrosis resulting from ureteral obstruction must be severe before the functioning renal tubular mass is reduced beyond a critical point to an inadequate amount Similarly, it may be assumed that prolonged reabsorption and excretion of urinary products do not lead to deterioration of the kidney

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4 Depletion of potassium results mainly from acidosis and the loss of secretions with mucus per rectum

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URETERAL SUBSTITUTION USING VESICAL MUCOSAL GRAFTS IN NONREVERSED SEROMUSCULAR ILEAL SEGMENTS *

A Preliminary Report

LEONARDO S J MARTIN, JAMES H DUXBURY, AND
WYLAND F LEADBETTER

The utilization of intestinal segments as ureteral substitutes has been popularized in recent years and this appears to be the most feasible substitute to date Experimental efforts to replace the ureter using metallic tubes blood vessel grafts peritoneum, tubes of mesentery, skin, fallopian tubes and muscular wall proved to be inadequate [1 2]

The anatomic proximity of the isolated ileal segment with its intact mesenteric pedicle to the urinary tract makes it a convenient graft material to replace the ureter Even when isolated it still possesses an excellent vascular supply and vigorous peristaltic contractions Although this appears to provide a satisfactory conduit it has however presented a number of objections when compared with the normal functioning ureter The objections have been those of absorption of excretory urinary constituents mucus secretion stasis of urine persistent infection, marked vesicoureteral reflux and with the passage of time the probable contribution of these factors to the development of progressive renal disease

This preliminary report undertakes to describe the technique and results of utilizing vesical mucosal patch grafts in narrowed nonreversed seromuscular ileal segments as a ureteral substitute

METHOD

Using 12 mongrel dogs under intravenous nembutal anesthesia, the approach was made via a midline abdominal incision The terminal ileum was brought through the wound and a segment 20 cm (or 35 cm if bilateral substitution was to be done) was isolated between clamps The length of this segment was varied according to the size of the dog and the corresponding distance between the kidney and the bladder Intestinal continuity was restored by end to-end anastomosis with a continuous Connell stitching using 4 0 chromic catgut with atraumatic needle and reinforced with interrupted 4 0 silk sutures down to the vascular pedicle of the isolated loop The wound and intestines were walled off, except for the isolated loop (Fig 1A) which was brought through the wound and turned upward and laid on saline packs The isolated ileal loop was then opened longitudinally along its antemesenteric border (Fig 1B) This permitted the segment to lie on the abdomen as a sheet of tissue with mucosal surface exposed Excessive blood loss was prevented during this part of the procedure by occluding the vascular pedicle with a serrefine clamp and released at 30 minute intervals to insure viability of the segment The mucous membrane was next stripped off bluntly in one of two ways either mucosa alone or mucosa and submucosa Peyer s patches

* From the Department of [illegible] Massachusetts This study was aided by [illegible] With the technical assistance of Drs W [illegible] partment of Radiology

were scraped off with a knife blade The denuded ileal segment was then narrowed on both sides decreasing its width to 1 5 cm (Fig 1C)

The urinary bladder was next exposed and was partially distended with sterile saline solution A longitudinal incision was made in the anterior wall and the submucosal surface made to bulge into the wound (Fig 1D) An adequate amount of mucosa was then separated from the bladder wall by sharp and blunt dissection and excised A small mushroom catheter was placed in the bladder through a separate stab wound The bladder was then closed in layers The freed vesical mucosa was cut into strips 3 cm in length and 1 5 cm in width (Fig 1E) and transplanted with the transitional celled surface facing the lumen to the denuded ileal segment The strips were placed 1 cm apart and held by sutures of 5 0 chromic catgut at their corners (Fig 1F) The ileal segment was now fashioned into a tube and snuggly sutured around a No 8 polyethylene splint to maintain its diameter The edges were sutured together with a continuous suture of 1 0 chromic catgut which approximated the muscular and mucosal layers accurately (Fig 1G) Occasional reinforcing interrupted sutures of the same material were used

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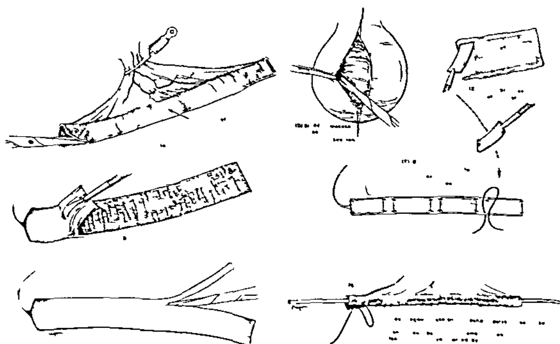


Fig 1 A Segment of terminal ileum divided at antimesenteric border
B Ileal segment is laid open and the mucosa stripped off
C Ileal segment narrowed on both sides
D Urinary bladder distended the muscle layer incised and the mucosa made to bulge and adequate mucosa excised
E Free bladder mucosa cut into 3 cm long and 1¼ cm width strips
F Bladder mucosa strips transplanted to narrowed ileal segment and held by stitches at its corners
G Ileal segment is fashioned around a polyethylene tube

URETERAL SUBSTITUTION USING VESICAL MUCOSAL GRAFTS IN NONREVERSED SEROMUSCULAR ILEAL SEGMENTS *

A Preliminary Report

LEONARDO S J MARTIN, JAMES H DUXBURY, AND WYLAND F LEADBETTER

The utilization of intestinal segments as ureteral substitutes has been popularized in recent years and this appears to be the most feasible substitute to date Experimental efforts to replace the ureter using metallic tubes, blood vessel grafts, peritoneum, tubes of mesentery, skin, fallopian tubes, and muscular wall proved to be inadequate [1 2]

The anatomic proximity of the isolated ileal segment with its intact mesenteric pedicle to the urinary tract, makes it a convenient graft material to replace the ureter Even when isolated, it still possesses an excellent vascular supply and vigorous peristaltic contractions Although this appears to provide a satisfactory conduit it has, however, presented a number of objections when compared with the normal functioning ureter The objections have been those of absorption of excretory urinary constituents, mucus secretion, stasis of urine, persistent infection, marked vesicoureteral reflux and with the passage of time, the probable contribution of these factors to the development of progressive renal disease

This preliminary report undertakes to describe the technique and results of utilizing vesical mucosal patch grafts in narrowed, nonreversed seromuscular ileal segments as a ureteral substitute

METHOD

Using 12 mongrel dogs, under intravenous nembutal anesthesia, the approach was made via a midline abdominal incision The terminal ileum was brought through the wound and a segment 20 cm (or 35 cm if bilateral substitution was to be done) was isolated between clamps The length of this segment was varied according to the size of the dog and the corresponding distance between the kidney and the bladder Intestinal continuity was restored by end to end anastomosis with a continuous Connell stitching using 4 0 chromic catgut with atraumatic needle and reinforced with interrupted 4 0 silk sutures down to the vascular pedicle of the isolated loop The wound and intestines were walled off, except for the isolated loop (Fig 1A) which was brought through the wound and turned upward and laid on saline packs The isolated ileal loop was then opened longitudinally along its antemesenteric border (Fig 1B) This permitted the segment to lie on the abdomen as a sheet of tissue with mucosal surface exposed Excessive blood loss was prevented during this part of the procedure by occluding the vascular pedicle with a serrefine clamp and released at 30 minute intervals to insure viability of the segment The mucous membrane was next stripped off bluntly in one of two ways either mucosa alone or mucosa and submucosa Peyer's patches

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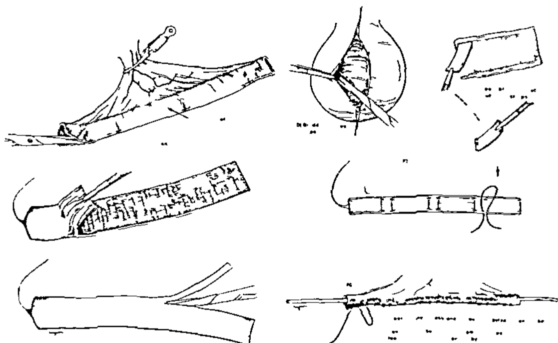


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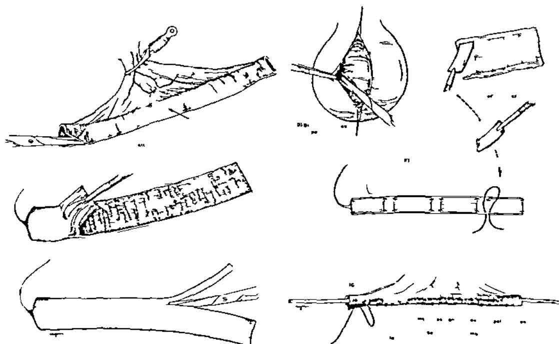


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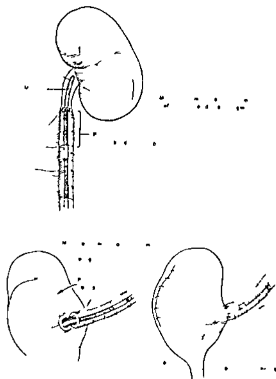
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E Free bladder mucosa cut into 3 *cm long* and 1¼ cm width strips

F Bladder mucosa strips transplanted to narrowed ileal segment and held by stitches at *its corners*

G Ileal segment is fashioned around a polyethylene tube



H Ureteral splint is inserted in the pelvis and a circular mucosa to mucosa anastomosis was done

I (a) Mucosa to mucosa anastomosis of opposite end of graft and posterior wall of bladder

I (b) Ureteral splint exited through the urethra

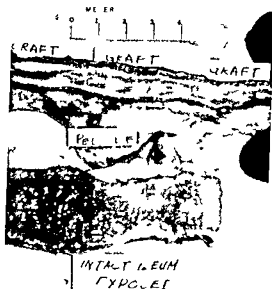


Fig 2 Luminal exposure of the ureteral ileal segment as compared to the exposed intact ileum Note grafted and nongrafted areas blending with each other

5 0 chromic catgut (Fig 1 I) The distal end of the segment was implanted into the posterior wall of the bladder by excising a ring of muscularis corresponding to the diameter of the new ileal segment and anastomosing vesical mucosa to vesical graft mucosa (Fig 1 Ia) The ureteral splint was brought out through the urethra in females (Fig 1 Ib), and through a cystostomy tube in males The posterior peritoneal edges were approximated, thus extraperitonealizing the ileal segment except for the proximal portion of the vascular pedicle whose edges were also sutured to the posterior peritoneum to prevent internal herniation Penrose drain was used retroperitoneally when necessary and the abdominal wound was closed in layers

RESULTS

All animals appeared well after operation Ureteral splints were removed in 2 weeks Four dogs with only the ileal mucosa removed were sacrificed at 1 week 2 4 and 8 weeks Gross appearance of these segments and kidneys appeared healthy The vesical mucosal patch grafts and the denuded spaces between them were distinctly evident at 1 and 2 weeks, and hardly distinguishable at 4 weeks (Fig 2) Microscopic study showed that vesical mucosal grafts were well fixed to the submucosal lining of the ileal segment The denuded spaces between mucosal grafts were overgrown by urothelial cells in 2 weeks and piling up to 3 or more cell layers at 4 weeks (Fig 3) Sections at the pelvioileal junction and vesicoileal junction showed an uninterrupted



Fig 3 A high power magnification of regenerated uroepithelium

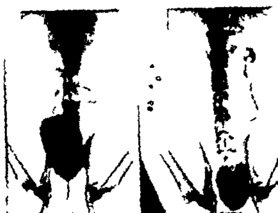


Fig 4 Delayed cystogram and intravenous study at 2 months after operation Note no vesicoureteral reflux and normal upper urinary tract

urothelial lining There was no evidence of ileal gland regeneration in these segments even at the end of 2 months

Cystogram studies in 4 dogs showed no reflux even when the bladder was well distended Intravenous pyelograms obtained at 1 and 2 months revealed good function and patency of the segments with no pooling of dye in the segment Figure 4 shows a delayed cystogram and intravenous study at 2 months

Cinefluoroscopic study of the peristaltic activity of the new ureter revealed vigorous peristalsis

Urine specimens were grossly blood tinged and cloudy at 1 to 4 weeks, but were grossly and microscopically clear of mucous material at 5 weeks after operation Blood chemistry study revealed elevated nonprotein nitrogen during the first month receding to normal level at the sixth week after operation Sodium potassium chloride and carbon dioxide level in the blood remained within normal limits

DISCUSSION

The remarkable proliferative capacity of uroepithelium when transplanted to a new site was demonstrated in this study It showed itself capable of rapid growth[8] from the vesical mucosal graft margin, and healing was completed by the end of 2 weeks with 2 or more cell layers in a denuded surface 1 cm in length

This combination of vesical mucosal grafts and narrowed nonreversed seromuscular ileal segments may provide the ideal ureteral substitute for the following reasons 1) the segment retains its vascularity and peristalsis, 2) the segment nourishes a new epithelial lining which is nonabsorbing and nonsecreting 3) stasis of urine is avoided and reflux discouraged, 4) the epithelial lining is capable of sterilization

Four dogs are now being observed for 8 months and appear to be *normal in* all respects This will be dealt with in a separate communication

SUMMARY

A new technique has been proposed to satisfy the requirements of an ideal ureteral substitute, utilizing a combination of vesical mucosal patch grafts and nonreversed seromuscular ileal segments The experimental results cited

using this method indicate that this technique may provide the ideal ureteral substitute

REFERENCES

1. Melnikoff, A E: Rev Clin Urol, *1* 601, 1912
2 Sanders, A R, and Schein, C J J Urol, *75* 639, 1956
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